

Descriptive study on Dental pulp stem cells in regenerative dentistry current findings and future perspectives

By

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Abstract

Dentinal repair in the postnatal organism occurs through the activity of specialized cells, odontoblasts that are thought to be maintained by unclear prelude population associated with pulp tissue. Mesenchymal stem cells (MSCs) live-in bone marrow are one of the most studied and clinically major populations of adult stem cells. In this study the researcher addressed the present state of knowledge regarding these cells, their properties, origins, locations, functions and potential uses in tooth tissue engineering and restore. However, the ultimate therapeutic use of hASCs requires that these cells and their derivatives maintain their genomic stability. To explore cytogenetic integrity of cultured human dental stem cell (hDSC) lines, the researcher analyzed four expanded hDSC cultures using classical G banding and fluorescent in situ hybridisation (FISH) with X chromosome specific probe. Our preliminary results revealed that about 70% of the cells exhibited karyotypic abnormalities including polyploidy, aneuploidy and ring chromosomes. The heterogeneous spectrum of abnormalities indicates a high frequency of chromosomal mutations that continuously arise upon extended culture. These findings emphasize the need for the careful analysis of the cytogenetic stability of cultured hDSCs before they can be used in clinical therapies.

Keywords: *Dental pulp, immunogenicity, stem cells ,regenerative dentistry.*

1. Introduction

Teeth are exclusive structures that contain a exclusive material (the enamel) that is highly conserved in the fossil record. Tooth origins have been return to the earliest vertebrates where skin dentils of died out fishes are thought to go into the oral cavity as jaws evolved. Teeth are only originate in vertebrates and their evolution is believed to be associated with the impression of the neural crest.

Mice as the most commonly used experimental animal model, do not advance canine and premolar teeth; substitute the incisors and the molars are separated by a region devoid of teeth called the diastema but humans have four incisors, two canines, four premolars and six molars in each jaw. Present replacement tooth methods such as tooth implantation use synthetic materials. Other possibilities include autotransplantation and subsequent reshaping. All these methods have been used in dentistry for many years, but have never been acceptable for some reasons such as cosmetic or physiological.

A large number of studies indicate the presence of stem cells in different tooth areas. They present a different potential for multilineage differentiation but undoubtedly are potential sources of cells for dental tissue repair and tooth tissue engineering. The result of their properties and to what expanse they can be used remains one of the greatest challenges in the dental field in the coming years.

So, in present study ,the researcher discusses a compact review of the main dental stem cell populations and their current and future perspectives in repairing lost dental tissues or even creating a new biological tooth.

Tooth stem cell

Teeth are organs that present unusual features. Generally, they are formed by the hardest tissue in the body and their soft tissue is completely surrounded by an external layer of hard tissue. According to these features, teeth have limited potential to respond after an injury and so, an inflammatory process will end up in pulp tissue necrosis. On the other hand, the aging process of the pulp tissue results in the formation of pulp stones followed by intensive deposit of collagen fibres and with reduction in pulp cell numbers. Therefore, dental pulp has no constant repair ability and will react only after damage with a limited type of response. In contrast to other parts of the human body.

Regarding this matter, it is not clear why cells related to the maintenance of the cell numbers and with a clear function related to injury response will be present in dental tissues, in particular in the pulp. Even the well known ability to form odontoblasts may be just a natural possession of a pulp fibroblast that does not show other stem cell properties such as capacity for self-renewal. But, the peculiar characteristics of the dental stem cells show that the propagation rates of multi-colony-derived DPSCs, SHED, and PDLSC are 30, 50 and 30% superior when compared with BMSSCs and the dental cells also show better immune regulatory action than bone marrow cells.

According to Baume(1980) and Smith et al.(1995)the crowns of human teeth consist of enamel, dentin, and dental pulp tissue. During tooth growth ameloblasts form enamel and odontoblasts produce primary dentin. After tooth eruption, ameloblasts vanish from the surface of the enamel, enamel formation ceases to take place naturally in vivo. In contrast, odontoblasts along the internal surface of the dentin inside the pulp chamber, continue to deposit dentin matrix to form secondary dentin throughout life. In addition to secondary dentin, odontoblasts can form tertiary dentin in response to several stimuli, such as mechanical, chemical, and/or bacterial stimulation (Cox et al., 1992; Kitamura et al., 1999; Smith et al., 1990, 1994).

As (Ruch, 1998; Sveen and Hawes, 1968) pointed out even when odontoblasts have been injured, the reparative dentin can be formed in the dental pulp to protect against further disruption of the pulp tissue. This reparative dentinogenesis has been thought to be mediated by newly generated odontoblasts that seem to arise from dental pulp tissue.

These results have led to the assumption that odontogenic progenitor cells or stem cells may exist in dental pulp tissue (Butler et al., 1997; Ruch, 1998; Sveen et al., 1968). As some researchers such as Buurma et al., 1999; Couble et al., 2000; Kuo et al., 1992; Shiba et al., 1995; Tsukamoto et al., 1992) have been mentioned pulp tissue contains proliferating odontoblast-like cells and that these cells are capable of forming mineralized nodules in vitro The cells isolated from dental pulp appear to have a limited capacity to differentiate into odontoblast-like cells and an inability to differentiate into other cell types such as adipocytes or neurons.

More recently, Teeles and his colleagues established that proliferating odontogenic precursor cells appear to be mobilized from blood vessels to sites of damaged pulp or dentin tissue (Teeles et al., 2005). Collectively, these studies describe the presence of preodontoblast cells present in dental pulp tissue, leading to speculation of the existence of putative dental stem cell populations.

As (Gronthos et al., 1994, 1996, 2003; Owen et al., 1988; Pittenger et al., 1999; Prockop, 1997; Simmons et al., 1991; Sonoyama et al., 2005) expressed mesenchymal stem cells were first isolated from bone marrow (bone marrow mesenchymal stem cells; BM-MSCs); they are a population of multipotent postnatal stem cells.

One of the most important characteristics of BM-MSCs is their capacity to form single-cell-derived colony clusters called colony forming unit-fibroblast (CFU-F) in vitro (Friedenstein, 1976; Friedenstein et al., 1970).

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According to Shi and Gronthos(2003) stated that accumulated knowledge regarding the phenotypic characteristics of BMMSCs has permitted us to isolate putative stem cell populations from the dental pulp of human third molars (dental pulp stem cells; DPSCs) and deciduous teeth (stem cells from human exfoliated deciduous teeth; SHED), which exhibit properties similar to those of BM-MSCs. Stem cells in dental pulp were found to reside in a specific perivascular microenvironment, where they are quiescent and maintain their basic stem cell characteristics including a self-renewal capacity and undifferentiated status. This specific microenvironment is called the “stem cell niche” (Bianco and Robey, 2001; Doherty et al., 1998; Fuchs et al., 2004; Moore and Lemischka, 2006).

In order to identify the factors that preserve the “stemness” of cultured mesenchymal stem cells any separation of mesenchymal stem cells must attract attention to their niche microenvironment which slowly drop their stem cell-like features after ex vivo growth.

Dental stem cells expand is produced during nervous system development and giving them the capability to distinguish into neural cell lines.

Humans during their lifetimes have two sets of teeth, baby and adult sets, , so “all we are trying to do is copy nature and give the person the third option to re-grow their teeth.” And if the tooth is deformed, it can be excluded and a new one put into place. The statistics on tooth loss are a bit staggering: 7 out of 10 adults age 35 to 44 have lost at least one tooth and a quarter of those aged 65 or older (or about 20 million people) have lost all their permanent teeth. On the other hand , side effects from medications can impact oral health like conversing properties of the saliva that cause fight bacterial development. According to a recent study ,raised tooth loss leads to deprived dietary habits, , which leads to secondary health effects. Stem cells has been gotten from baby teeth with embryonic-like features and are low in immunogenicity.

In order to the treatment of Parkinson’s disease, cranial bone repair, heart disease, spinal cord ,diabetes, and brain injuries, , and root formation , mesenchymal stem cells (that are detected in dental pulp and other tissues) are being used in research. Dental stem cells have been induced to form bone, nervous tissue, and beta islet cells, which produce insulin. No regenerative treatments currently use dental stem cells, but animal studies are exploring regenerative applications.

Most research currently study the host’s own stem cells through extrinsic growth factors to form bone by attracting stem cells and inducing them to become osteogenic cells. Allogeneic stem cells are used in sinus lifts and periodontal defect repair to reduced the time needed for bone arrangement and may ultimately propose a possible replacement for autologous bone grafts. The sources of autologous dental stem cells may be baby teeth, wisdom teeth, or other extracted healthy permanent teeth. These can be banked for use by the donor in the future. This would eliminate the risk for infection and the need for anti-rejection drug use. Stem cells has gotten from baby teeth and developing wisdom teeth have pluripotential and embryonic-like characteristics and are low in immunogenicity.

So far, teeth have been regenerated in mice and monkeys, and clinical trials with humans are underway, but whether the technology can generate teeth that are nourished by the blood and have full sensations remains to be seen. Teeth present a unique challenge for researchers because the stem cells must be stimulated to grow the right balance of hard tissue, dentin and enamel, while producing the correct size and shape.

Having a full set of functional teeth is increasingly important as an aging population seeks to maintain an active lifestyle. Dentists are at the front line of the increased demand for perfect teeth. A 2009 nationwide survey by NSU revealed that 96% of the dentists polled expected stem cell regeneration to dominate the future of dentistry. Additionally, more than half predicted that the technology would be available within the next decade.



In mice, stem cells grew into a tooth (in green) that had similar properties to natural teeth. Although the promise of stem cell therapies remains to be realized, there's little doubt that researchers at NSU and around the world will continue in their efforts to use stem cells for regenerative medicine.

Current Practice and Research

It is still unclear how the different dental stem cell populations behave and function and indeed the extent to which any of those can be considered to be mesenchymal stem cells rather than progenitors remains controversial. However, the potential of these cells as being 'bankable' is unique since children lose up to 20 teeth naturally as the permanent dentition erupts, and most adults lose teeth at some point of life. In addition, the accessibility of the pulp chamber in an adult is simple and straightforward and does not result in morbidity to the patient. Even the access to the root apical papilla area is uncomplicated with the removal of the third molars. Therefore, teeth are undoubtedly a promising source of autologous stem cells for tissue engineering. The majority of craniofacial cartilages and bones are formed by neural-crest-derived mesenchymal cells that after migration will further differentiate into neural, pigmented and smooth muscle cells, craniofacial cartilage and bone. In this context, a naturally exfoliated deciduous tooth or other dental tissues are similar in some ways to the umbilical cord, containing stem cells that may have potential to repair damaged structures in the craniofacial area or in other parts of the body.

Reconstruction of Bio-tooth from DPSCs Tooth loss is a common and frequently occurring disease in the aging populations that adversely affects the masticatory efficiency, language function, facial esthetics, and psychological health. In the developed countries, an estimated 7% of people have lost one or more teeth by age 17. After age 50, an average of 12 teeth have been lost. World Health Organization (WHO) databanks demonstrate that dental caries is still prevalent in most countries worldwide (100% incidence in some populations); severe periodontal diseases which may result in tooth loss are estimated to affect 5–20% of most adult populations, and the incidence of complete edentulism has been estimated between 7% and 69% internationally. To treat these missing teeth, current approaches mainly focus on the artificial materials or non-biological implants that can unavoidably reduce the quality of life due to their limited physiological functions and sometimes elicit an immune rejection. Bio-tooth is thought to be a kind of biological tooth that can be re-integrated into the jaw and perform the normal functions of a natural tooth including the regenerative ability in case of injury. Using the principle of epithelial-mesenchymal interactions to guide the tooth regeneration has become a common strategy in dental tissue engineering. Many studies have demonstrated that the bio-tooth can be reconstructed by dental cells recombined with or without scaffolds, by pre/post-natal dental cells, and even by nondental cells. Nakao et al. have demonstrated that bioengineered incisor tooth germs can be reconstituted using completely dissociated

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dental epithelial and mesenchymal cells in a three-dimensional collagen gel. These bioengineered tooth germs can replicate the embryonic tooth organogenesis and develop into the whole incisor in vitro or in the dental alveolus of adult mice. Furthermore, Ikeda et al. have proved that these bioteeth in the alveolar bone can perform the functions of a natural tooth including the eruption, occlusion and mastication, which highlights a new exciting prospect of bio-teeth in future clinical applications.

Sonoyama et al. have proposed the stem cell-based bio-root regeneration by integrating several approaches together including stem cell techniques, biomaterials, and crown restoration. The recombinations between SCAPs/HATCP and periodontal ligament stem cells (PDLSCs) can bring about the formation of bio-root/periodontal complexes, which can support a porcelain crown to restore the normal masticatory and aesthetic functions. Previous work has revealed that DPSCs pellet reassociated with adult rat apical bud cells can form a typical crown-like structure in vivo containing distinctive ameloblast layer, enamel, dentin, predentin and odontoblast layer. However, no root-like structure has been observed during DPSCs-based tooth regeneration, due to the complex mechanism inherent in root development. Recently, many types of cells including DPSCs, SHEDs, and SCAPs have been successfully reprogrammed into iPS cells which hold a great promise for regenerative medicine. These dental iPS cells express the marker genes that characterize embryonic stem cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Our unpublished data further reveal that the marker genes of ameloblast lineages (ameloblastin and amelogenin) are significantly up-regulated in DPSCs-derived iPS cells, as indicated by real-time RT-PCR analyses. Thus, whether we can use these dental iPS cells to make a bio-tooth is still full of uncertainty and challenges. Firstly, autologous DPSCs are isolated from the patient's own dental pulp and amplified in vitro. Secondly, iPS cells are generated by driving four genes (c-Myc/Klf4/Oct4/Sox2) into DPSCs.

These dental iPS cells can give birth to dental epithelial cells under suitable conditions because of their pluripotent properties. Then, these iPS-derived dental epithelial cells are re associated with autologous DPSCs and subsequently transplanted in vivo for temporary incubation to produce a bio-tooth. Finally, bio-tooth germ or whole bio-tooth can be transplanted into the patient's jaws for continuous growth and eruption. Mesenchymal stem cells isolated from adult human dental pulp and periodontal ligament have been known to have the potential to differentiate into odontoblasts, cementoblasts, adipocytes, chondrocytes, and myoblasts (Seo et al, 2004; Shi et al, 2005; Chang et al, 2006). Summary For clinical treatment of tooth defects and tooth loss, nonbiotechnological approaches, such as the use of prostheses and implants, have generally been employed. Dental regenerative therapies which restore or replace defective teeth using autologous explants are being investigated using current understandings of developmental biology, stem cell biology, and regenerative medicine. Recently, dental tissue stem/progenitor cells, which can differentiate into dental cell lineages, have been identified in both impacted and erupted human teeth, and these cells can be used to regenerate some dental tissues. Tissue engineering using scaffold and cell aggregate methods may also be used to produce bioengineered teeth from dissociated cells for therapeutic applications of whole tooth replacement. Recent breakthroughs in single cell manipulation methods for the reconstitution of bioengineered tooth germ and the investigation of in vivo development of artificial tooth germ in the adult oral environment have been reported.

These researches and developments will ultimately lead to the realization of dental regenerative therapies for partial repair by stem cell transplantation and for whole tooth replacement using bioengineered tooth germ. It is becoming increasingly clearer that this conceptual approach to therapy, named "regenerative dentistry," will have its place in the clinical practice of dentistry in the future. cell-based regenerative dentistry. In most organs, such as the blood, skin, liver, and brain, there are tissue stem/progenitor cells that appear to have limited abilities to differentiate and perform maintenance and restoration of these organs. It has been anticipated that dental tissue stem/progenitor cells would be found in adult tissues because spontaneous repair of dentin and periodontal ligaments has often been observed. Dental tissue stem/progenitor cells would be particularly useful in dentistry for the development of cell transplantation

therapies for the repair of damaged dentin and for periodontal disorders, and the cells are therefore currently the subjects of extensive research. Somatic stem cells which can differentiate into odontoblasts have been identified in pulp tissue of human permanent teeth, exfoliated deciduous teeth, and impacted teeth. In addition, the side population fraction, based on the efflux of fluorescent dye Hoechst 33342, of human dental pulp cells, and the periodontal tissue stem cells derived from human extracted teeth can partially regenerate dentin and periodontal tissue by cell transplantation into surgically created defects in adult teeth. Stem cell-based regenerative therapies certainly hold much potential in the treatment of medical and dental conditions. Indeed, many patients around the world have already benefited from such therapies. However, the decision to incorporate stem cell-based therapies into routine clinical dental practice requires careful analysis of the risks and benefits associated with the procedure. For example, while the potential benefits of stem cell transplantation for patients with hematological cancer tend to outweigh the risks, the same may not be necessarily true for their use in dental procedures. It is unquestionable that the processes of storage and expansion of stem cells in laboratory settings, as well as the transplantation of these cells back to the patient, carry certain risks. There is a risk of transformation of the stem cells, and there is also a risk of unwanted contamination of these cells with pathogens during these procedures.⁷⁰ While these risks are relatively small, they exist and cannot be ignored. Indeed, it is certainly imperative that patients undergoing such procedures with stem cells in investigative or clinical settings are made fully aware of such risks.

While the use of stem cells brings many new therapeutic opportunities, and perhaps will allow for the treatment of dental conditions that are untreatable with today's materials and procedures, one must proceed with caution. It is imperative for clinical procedures with stem cells to be supported by solid basic and translational research. It will be only through rigorous research that the full extent of the potential risks involved in the use of these cells will be understood, and the means to prevent (or overcome) them will be discovered. It will also be only through research that the biology of tooth-related stem cells and the therapeutic potential of these cells will be better understood. Progress will depend on the collaboration between clinicians and researchers from diverse fields (e.g., biomaterials, stem cell biology, endodontics) working together toward the goal of developing biological approaches to regenerate dental and craniofacial tissues. It should be said at the present time researchers anywhere in the world, do not have a method for three-dimensional organs culture in vitro the outside the body. So creating technologies are important for making organs in a laboratory bioengineered replacement organs for regeneration therapy . Another group of Japanese bio engineers have succeeded in growing a tooth from cells transplanted into mice. Through an alternative technique that can shoot much faster than alternative organ ,biologists succeeded to combine cells needed to form teeth with real teeth to make a rat kidney transplantation.

According to Professor Takashi Tsuji, director of the project in comparison with the previous method, cause to save the tooth for 10 days. He stressed that the first step toward the goal of replacing damaged tissues and organs that produce original has vanished and didn't receive to the stage where it could be used for human beings. Scientists say that mouse feel pain and irritation because the performance of his teeth were like real teeth. The researchers hope that one day they by using engineered active organs can produce organs to replace damaged by disease or injury.

Anthony Smith from» Birmingham University in United Kingdom said: Some researchers added, the protein to foster the growth of stem cells derived from dental pulp cells after transplantation and some researchers aim to develop new healthy pulp inside of teeth by using stem cell technology to control the inflammation that causes the growth of new teeth .Nevada Dental School researchers through out the stem cells within the bone marrow of the patient's teeth or teeth that are actually produced and replace the tooth in the patient's mouth grows both in vitro..

Future perspectives on dental

Previous research in dental regenerative medicine indicated the possibility of not only stem cell transplantation, but also organ substitution, before than for other organs. Nowadays, it is necessary to discover useful and easily available source of a patient's own cells to keep away from rejection. It has been shown that non-dental tissues such as adult bone marrow presents useful cell sources. Also, it will be important to clarify the mechanisms of tooth morphogenesis to control the size and shape of bioengineered teeth. The bioengineering technologies developed for tooth regeneration will make substantial contributions to understanding the developmental process and will encourage future organ replacement by regenerative therapies in a wide variety of organs, such as the liver, kidney, and heart. In importance in the advancement of regenerative therapies more research to coordinate advances in various fields, such as materials science, clinical medicine, biology, and chemistry will increase.

Stem cells are being applied to dental implants to form a ligamentous attachment between the implant and bone. The ability to regrow dental pulp is expected to be developed soon based on obvious impact on endodontics practice. Dental stem cells may be used to repair cardiac muscle damaged in a heart attack or ischemic heart disease. The roots of teeth have been regrown in animal models, and so people may eventually be able to regrow their own tooth roots to replace implants. Currently many teeth are being discarded as medical waste.

2. Conclusion

In conclusion, the understanding of dental stem cells and tooth repair is still evasive and further study, especially using *in vivo* approaches are necessary to understand and better characterize the different populations of dental stem cells. The field of stem cell-based regenerative dentistry is not simple by nature. There is currently a lack of systematic studies that are aimed at characterizing opposing chromosomal changes in cultured ASCs during the time. But, the reality of mosaicism and accumulation of karyotypic abnormalities within cultured cell subpopulations have been reported. Research in this field is still in its early stages, according to researchers at least a decade must wait until the new technology of tooth outbreak develop. Stem cell-based dental tissue regeneration is a new ground that has the potential to pave the way. Its future will depend on the accepting of the biology of the cells that will be used to redevelop tissues,. One of the major obstacles that must be defeat by sufficient source of stem cells for use in human mouth. Researchers are trying to keep their teeth for patients.

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