

DENTAL STEM CELL SOURCES AND THEIR POTENTIALS FOR BONE TISSUE ENGINEERING

Dental kök hücre kaynakları ve kemik doku mühendisliğinde kullanılma potansiyelleri

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Received: 28/05/2014

Accepted: 05/11/2014

ABSTRACT

Tissue engineering arouses excitement in all medical fields that deal with bone healing. The ultimate aim of these approaches are to shorten the healing process, obtain highly differentiated functional tissues and eliminate the need for a second surgical site required for autogenous bone grafts. Mesenchymal stem cells have been increasingly used in the experiments which were conducted in these fields and the results are promising. Dental stem cells have come to the forefront both because of their relative ease of access and also their superior characteristics. This article investigates the importance of dental stem cells for bone tissue engineering and their regeneration potentials.

Keywords: Dental stem cells; bone tissue engineering; oral and maxillofacial surgery

ÖZ

Doku mühendisliği yaklaşımları, kemik iyileşmesi ile ilgilenen her tıp alanında heyecan uyandırmaktadır. İyileşme sürelerinin kısalması, daha kaliteli ve fonksiyonel doku elde edilmesi ve ikincil bir cerrahi sahaya gereksinimi ortadan kaldırması bu yaklaşımların nihai hedeflerindedir. Mezenşimal kök hücreler bu alandaki çalışmalarda giderek artarak kullanılmakta ve sonuçlar umut vermektedir. Dental kök hücreler de hem kolay temin edilebilmesi hem de üstün özellikleri sebebiyle ön plana çıkmaktadırlar. Bu çalışmada dental kök hücrelerin kemik doku mühendisliği yaklaşımları için önemi ve rejenerasyon potansiyelleri incelenmiştir.

Anahtar kelimeler: Dental kök hücreler; kemik dokusu mühendisliği; oral ve maksillofasial cerrahi

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Introduction

Healing and/or regeneration of the bone tissue is the main goal of oral and maxillofacial surgery practice. Particularly, reconstruction of bone defects following cyst and tumor surgeries, replacing the alveolar bone loss and maxillary sinus augmentations are frequently performed procedures which aim to create an adequate bone volume that allows an acceptable prosthetic rehabilitation. Autogenous bone graft continues to be the gold standard in clinical use (1). However, in parallel to advancements in tissue engineering studies, it can be foreseen that this situation would not last long. Besides, the complications that might occur during the harvesting of bone graft (morbidity, infection) and the occurrence of a secondary surgery site bring the need for searching alternative treatments. On the other hand, artificial materials such as allografts and xenografts have their own disadvantages which limit their clinical use (2). Bone tissue engineering approaches aim at obtaining intended functional tissue by using proper carrier and bioactive factors (3). Currently, studies are being performed to determine both ideal carrier and ideal stimulating factor. Prolonged healing times also pose an important problem for bone tissue research. Several studies showed that bone tissue healing can be achieved with cell stem supported bone tissue engineering approaches (4). Stem cell studies draw considerable attention since their discovery. Their characteristics to renew themselves and to differentiate into other cell types make them a promising source. It is possible to detect stem cells in normal tissue healing as well as in regeneration and repair following tissue damage (5). Stem cells can be found in any region of the human body including dental tissues. Those that have been isolated from dental sources so far are; dental pulp derived stem cells, stem cells derived from exfoliated deciduous teeth, periodontal ligament stem cells, apical papilla stem cells and dental follicle precursor cells (6). The aims of this review were to examine the stem cell-based tissue engineering approaches within the scope of oral surgery and to evaluate recent developments.

Embryonic Stem Cells

Embryonic stem cells are derived from the blastocyst inner cell mass between the 4th and 7th days following fertilization. This cell group demonstrates the highest potential to differentiate into all three

embryonic germ layers because of its totipotent (e.g. ability to differentiate into all cell types) characteristics. Embryonic stem cells are generally obtained by getting remaining fertilized cell mass after in-vitro fertilization (test tube baby method) with the approval of the patient. On the other hand, religious and ethical issues have been raised because of cloning studies, collection methods and the potential of these cells to form living organisms (7). Stem cells can form tumoral masses (teratoma) that contain all germ layers because of their high division and differentiation potentials. Therefore, before initiating routine clinical use of embryonic or mesenchymal stem cells, their controlling process and differentiation mechanism must be examined thoroughly. On the other hand, in 2007, an important study was performed for solving this problem. Takahashi *et al.* (8) achieved to obtain embryonic stem cell like cells by stimulating mature somatic stem cells and called them “induced pluripotent stem cells (iPS)”. These reprogrammed cells are similar to embryonic cells in terms of their antigenic surfaces, gene expressions, morphology and telomerase activity. This study that constitutes an alternative for the use of embryonic stem cells aroused excitement for further stem cell studies.

Mesenchymal Stem Cells

Mesenchymal stem cells can be found in mature tissues of stromal origin and constitute the base for connective tissue. Recently, they were isolated from several tissues of the body such as bone marrow, skeleton, heart muscle, tooth, liver, testicle, ovaries, skin, brain, blood and lungs (9). Mesenchymal stem cells can be obtained from almost every tissue and they do not form teratoma. However, their capacities are limited compared to embryonic stem cells. Because their numbers are relatively smaller and they must be cultured several times to multiply, their telomerase activities and differentiation potentials are weaker when compared to embryonic stem cells. Although they tend to differentiate into the tissue cells where they are present, they are still promising sources for bone tissue engineering (10).

Dental Pulp Derived Stem Cells (DPSC)

The isolation of dental pulp stem cells was done for the first time in 2000 by Gronthos *et al.* (11) and it is still a very important source. First DPSCs were isolated from an impacted third molar. Studies have

shown that the DPSC demonstrated high proliferation and differentiation potentials under stimulated micro-environmental conditions. When proper conditions are provided, it was found that DPSCs can differentiate into odontoblast, osteoblast, chondrocyte, myocyte, neurocyte, adipocyte, cornea epithelial cells and iPS cells (12, 13). DPSCs that were kept in laboratory environment for 2 years could form bone on acceptable levels in rats by using hydroxyapatite-tricalcium phosphate carriers. Therefore, in the future, it can be possible to store these cells under proper conditions (14). The application of dental stem cells to humans was tried by d'Aquino *et al.* (15) in 2009. DPSCs were obtained from the maxillary wisdom teeth of 17 volunteer patients who presented with bilateral bone resorption of the alveolar ridge distal to the second molar due to the impacted third molars on the cortical alveolar lamina, producing a defect without walls. Authors seeded cells onto a collagen sponge scaffold and used it to fill the defect site left by extraction of the mandibular third molars. Three months later, they reported that the alveolar bone showed optimal vertical repair and complete regeneration as assessed by clinical probing, radiography and histology. It was reported that DPSCs can differentiate into bone tissue very easily. Moreover, it was shown that in in vitro conditions, the differentiation potentials of DPSCs are better than bone marrow stem cells that are accepted as the current standard in stem cell studies (16). In the study that was conducted in 2011, Li *et al.* (17) showed that DPSCs in gelatin sponge carrier can differentiate into bone tissue cells even when they are placed ectopically. Authors have suggested that this could be a suitable factor in bone tissue engineering studies in terms of bone tissue regeneration and repair. DPSC is known as a promising source for stem cell-based regeneration, not only in dentistry and maxillofacial surgery, but also in different fields of regenerative medicine. It was shown that DPSCs could be beneficial in the treatment of myocardial infarction, nerve tissue regeneration, muscular dystrophy, cerebral ischemia and corneal regeneration (18-21).

Dental Follicle Precursor Cells (DFPC)

Dental follicle is a loose connective tissue that surrounds a developing tooth. It assumes an important role for the eruption by arranging osteogenesis and osteoclastogenesis (22). The fact that cement, bone and periodontal ligament tissues are originated from this

structure gives an idea about its potentials. Since it is a premise of periodontal tissue, it is thought that it can be a good source for periodontal regeneration. After the discovery of the presence of stem cells in dental follicle, the studies for elucidating their characteristics and potentials have increased in number. It was shown that these cells have a higher proliferation rate and osteogenic differentiation potential as compared to bone marrow stem cells (23). Honda *et al.* (24) proved the osteogenic potentials of DFPCs on rat calvarial defect model and reported that they can be a proper candidate for bone tissue engineering.

Stem Cells Derived from Apical Papilla (SCAP)

Apical papilla is present in teeth whose roots are not fully formed and it is loosely connected to the pulp. SCAP was isolated and used, for the first time in 2006, to form artificial tooth root with tissue engineering approaches. It was reported that SCAPs could be a suitable source in tissue engineering especially for artificially creating tooth root (25).

Between apical papilla and pulp resides a line rich with cells. Sonoyama *et al.* (26) showed that there are both DPSC and SCAP in this area and they carry different characteristics. In fact, it was reported that SCAPs could be a suitable source for cell-based regeneration by showing more successful proliferation characteristics as compared to DPSCs.

It is a known clinical situation that in permanent teeth whose development were not completed, apexification continues after protective endodontic treatment as a result of apical periodontitis or abscess (27). Although there are many theories regarding this process, it hasn't been clarified yet. However, this situation may be related to the SCAP activity. The primary odontoblasts that are formed with the effect of these cells after endodontic disinfection might cause the completion of root development (28).

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

While growth of permanent teeth continues, resorption can be seen in the roots of deciduous teeth. It was observed that the stem cells which are derived from the pulps of extracted deciduous teeth have different characteristics from DPSCs and they were therefore named SHED (29). These cells have demonstrated better replication and proliferation characteristics when compared to BMSC, PDLSC and

DPSC but cannot form complex pulp-dentin structures as DPSC can (30, 31). They have less mature cells in comparison to SHEDs and DPSCs (32). This situation might be explained with higher telomerase activity which leads to better replication and proliferation characteristics. In the study that was conducted to evaluate the osteoinductive potential of SHED, it was observed that they formed a dentine like skeleton in rats and they could differentiate into bone cells.

Seo *et al.* (33) reported that they have used SHED cells to successfully treat critical bone defects in rat calvarium and showed osteoinductive effects of these cells. Besides, since they express neural markers, SHED can also be a suitable source for neurologic regeneration. These cells can be easily obtained from extracted deciduous teeth (34). Therefore, “teeth-bank” may be a tangible option in the near future.

Periodontal Ligament Stem Cells (PDLSC)

Periodontal ligament is a connective tissue that resides between cement and alveolar bone and provides mechanic support for the tooth in alveolar socket by absorbing forces during chewing. Progenitor cells are present in periodontal ligament tissue that can differentiate into osteoblasts or cementoblasts. Stem-cell characteristics of these cells was first shown by Seo *et al.* (35) in 2004. In this study, it was also reported that under well-controlled culture conditions, PDLSCs might differentiate into cementoblast-like cells, adipocytes, and collagen-forming cells. When transplanted into immunocompromised rodents, PDLSCs showed the capacity to generate a cementum/PDL-like structure and contributed to periodontal tissue repair. Transplantation of these cells, which can be obtained from an easily accessible tissue source and expanded ex-vivo, might hold promise as a therapeutic approach for reconstruction of tissues destroyed by periodontal diseases. In another study it was reported that PDLSC which was produced in ex-vivo conditions and applied with fibrin sponge scaffolds could differentiate into bone tissue and has regenerative potency (36).

Based on previous findings regarding PDLSC, it can be stated that this cell line might be a potential candidate for tissue engineering and it can be useful especially in the repair of periodontal defects and artificial root formation (28).

Bone Tissue Engineering

Bone marrow engineering concept started in the midst of 1980s and interest in it has continued until today. The number of studies conducted in this field and accumulation in the literature has been rapidly increasing. Bone marrow engineering is a multidisciplinary field that aims to eliminate clinical disadvantages such as donor morbidity, limited graft volume, immune incompatibility and infection transmission. To achieve these goals, new carrier materials, growth factors and application techniques must be researched. Bio-medical engineers and clinicians have to collaborate in order to produce a suitable bone graft material and stimulating factors with which repair and regeneration can be achieved after bone tissue damage and atrophy (37). Conventional bone tissue engineering approaches consist of a number of basic concepts. These are; a biocompatible carrier design that is as similar to natural bone structure, cells that stimulate the formation of intended tissue type, providing a sufficient vascularization for suitable morphogenetic signals and development of the tissue. The structure that is transferred to living tissue should be osteogenic, be able to induce the formation of new blood vessels and there should be no blind spots where cells cannot reach (38). Although there is not an application or product that has been used in clinical practice, studies for ideal design of all these basic components that form bone marrow engineering have been continuing in a promising way. In order to generate new functional bone tissue with bone tissue engineering approaches, it is necessary to have a good understanding of stem cell, its biology, development and skeletal mechanics.

Conclusion

Easily obtainable, relatively inexpensive and biologically effective materials are required to increase the applicability of bone tissue engineering in clinical practice. Based on their superior differentiation and proliferation characteristics, dental stem cells are attractive sources that holds promise for future treatment of patients.

Source of funding
None declared

Conflict of interest
None declared

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