

## Stem cells are the hope of modern stomatology

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### ABSTRACT

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**Introduction:** Stem cells are often hailed as the medicine of the 21<sup>st</sup> century. They provide us with potential tools to effectively counteract not only diseases, but even aging. For stomatology, stem cells are the technology of the future in the regeneration of the periodontium and pulp, and dental replantation and transplantation.

**Materials and methods:** On the basis of a literature review, the previous achievements and potential capabilities of stem cell therapy were discussed, focusing on dental applications.

**Conclusions:** The paper discusses the modulation of stem cells and their therapeutic potential and

capabilities. The presence and properties of stem cells in the pulp of human deciduous and permanent teeth, the periodontal membrane and the dental sac are also discussed. The results of the studies conducted by the cited researchers are promising and give hope for the development of regenerative and restorative processes of the dental and periodontal tissues.

**Summary:** In the future, stem cells obtained from primary and permanent teeth deposited in special dental banks will be able to prevent the degradation of periodontal tissue, or even heal the teeth.

**Keywords:** Stem cells, stomatology

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## INTRODUCTION

Stem cells are often hailed as “medicine of the 21<sup>st</sup> century” or “the key to longevity”. With stem cells, in the near future scientists will be able to slow down the process of aging and significantly extend the human life span. Stem cells serve as a reservoir of cells with various degrees of development and differentiation, which have the ability to regenerate damaged organs and tissues. Their characteristic feature is the ability to regenerate and transform into specialized tissues and organs, thus making stem cells a promising solution for developing therapies against conditions such as hepatic diseases, myocardial infarction, stroke, or Parkinson's disease [1].

Two basic types of stem cells are distinguished: totipotent embryonal cells, which can differentiate in any type of cell; and somatic cells of limited differentiation potential [2]. The mechanisms controlling the differentiation of those cells are not yet understood [3].

Stem cells have also been found in dental and periodontal tissue. Their properties and potential application in various fields of stomatology are being studied. Those cells can be found in embryonal dental development stages, as well as in the pulp of mature teeth. Cells participating in the activation of stem cells in the pulp (mesenchymal mucous tissue of the tooth) in the regenerative process of a damaged tooth are not only odontoblasts, which are dentinogenic cells, but also the cells located below them. Those cells are responsible for the regenerative processes of the odontoblasts and the dentin, and under certain conditions undergo divisions which recreate an undifferentiated cell; the undifferentiated cell is then able to synthesize the extracellular matrix [4]. Among the cells with features of stem cells isolated from dental and periodontal ligament tissue, the following groups are distinguished: dental sac stem cells, stem cells of apical papilla, stem cells from exfoliated human deciduous teeth, mature dental pulp stem cells, and periodontal ligament stem cells [5].

### Dental Pulp Stem Cells (DPSC)

The first isolated stem cells were DPSC, i.e. dental pulp stem cells from permanent human teeth [6]. They are found in the perivascular areas of dental pulp [4]. *In vitro*, those cells proliferate rapidly and their colonies form calcified structures. It has been demonstrated that DPSC grafted on a hydroxyapatite framework under the skin of a mouse produce dentinoid tissue containing type 1 collagen. DPSC are characterized by multipotency, as they are able to differentiate into osteoblasts, endothelial cells, and nerve cells [7-9].

Similarly to DPSC analysis, the research of stem cells from apical papilla (SCAP) have shown

that those cells also have the ability to differentiate into functional odontoblasts [10], which has confirmed the multipotent character of both those cell lines. One of the key advantages of stem cells obtained from dental pulp and apical papilla is their clinical availability.

For study purposes, cells from both cell lines are sampled from unerupted third molars, which begin to develop around age 6, when DPSC and SCAP are in the early stages of cellular differentiation [11].

Dental pulp and papilla are minced using a scalpel and then etched with a dispase and collagenase solution. After the etching stage has been completed, the cells are centrifuged and filtered, and placed in bottles with growth medium plus bovine serum, L-glutamine, penicillin and streptomycin. In order to grow homogeneous mesenchymal cells, and to eliminate leukocytes and hematopoietic cells before starting the experiment proper, the DPSC and SCAP underwent a screening process using antibodies and the magnetic cell sorting system (MACS). Next, the cells were sorted, incubated, washed in a reaction mix and centrifuged. The control cells were grown on a standard culture medium supplemented with bovine serum, L-glutamine, penicillin and streptomycin [12,13].

The culture medium of the study cells was enhanced with ingredients that stimulated mineralization. The factors responsible included dexamethasone,  $\beta$ -glycerophosphate and L-ascorbic acid [10,11,14,15].

The culture medium was replaced every three days. After the cells reached an 80% confluence, understood as the coverage of the culture dish by the cells, the culture was removed from the substrate and disseminated to further culture bottles. After 6 weeks of observation, the proliferative potential and the ability to form colonies was compared between the study group and the control group, and with respect to baseline.

It was observed that the cells in the baseline groups of both DPSC and SCAP lines underwent more rapid division than the cells from the analogous control and study groups after 6 weeks of growth. Thus, the proliferative potential of cells in the control groups of both cell lines was reduced. The analysis of proliferative potential of DPSC and SCAP confirmed the different proliferative potential of both cell lines, which resulted from different stages of maturity of the analyzed cell groups. It has been confirmed that after the primary dentin has been completely formed, the odontoblasts found in the dental pulp lose their activity and need to be replaced by newly differentiated progenitor cells originating from the perivascular niche of the dental pulp [16].

A slightly different role is played by progenitor cells originating from the apical papilla,

which serve as the source of primary odontoblasts with the capacity for producing primary root dentin [17]. Stem cells from human dental pulp and apical papilla from the same unerupted molar exhibit different proliferative potential. This indicates that the cells from the apical papilla constitute a much more flexible reservoir of stem cells, and retain a more primary character both in standard and stimulated culture conditions.

#### **Dental pulp stem cells (DPSC) and stem cells from human exfoliated deciduous teeth (SHED)**

Stimulated tissue regeneration with the use of stem cells is a dynamic process comprising two components: implanted cells and the culture environment [18]. Studies on the properties of DPSC and SHED completed thus far have confirmed that it is possible to apply stem cells from the dental pulp of permanent and deciduous teeth to a broad range of therapeutic procedures, with DPSC having a higher pro-odontoblastic potential.

The dental pulp is isolated from the lesion by the defensive response of a mature odontoblast, which produces secondary and reparative dentin in response to external physiological and pathological stimuli. A similar role is played by the dentin bridge, which forms after odontotropic medications have been applied to the dental pulp exposure site. As it turns out, the deficiencies of mineralized dental tissue are not regenerated; hence studies on the clinical application of DPSC and SHED are fully justified. Those studies focus on finding biomimetic agents containing chemotactic factors for dental stem cells, and creating biological material containing DPSC and SHED. The ability to use those cells in treating deep carious lesions, direct coverage of the dental pulp, and treating reversible pulpitis would make it possible to restore the continuity of the dentin in the form of a biological dental filling.

Currently, an important factor for the success of progenitor cell-based therapy is the ability to control the regeneration process of dentin, avoiding uncontrolled accretion thereof, which could obliterate the pulp cavity. Gronthos' studies on isolating dental pulp stem cells indicate that this cell line is able to produce single fibroblast-like cells that can form a colony. Under a microscope, DPSC appear similar to fibroblasts. They have an elongated shape with a centrally located nucleus and a well-developed coarse endoplasmic reticulum. Golgi's apparatus is situated near the nucleus, and the large number of secretory vesicles indicates the secretion capability of the cell [11].

DPSC have all the features of mesenchymal stem cells. Using epitopes, i.e. portions of antigen which bind directly to a free antibody, B-cell receptor or T-cell receptor, such as STRO1 (i.e. receptor presented by mesenchymal

cells) and CD146 membrane cofactor protein, has made it possible to identify the mesenchymal stem cells in dental pulp [19-22]. Studies of *in situ* hybridization and immunohistochemistry studies have determined the location of DPSC, which were found in dental pulp vascular niches. A specific property of DPSC is the ability to regenerate the pulp/dentin complex, which has been confirmed by the expression of the DSP protein – a single specific dentin marker. The specificity of this protein consists in the fact that it undergoes biosynthesis in the dentin in concentrations that are hundreds times higher than in other cells. A pulp/dentin complex was formed in an experiment comprising the implantation of DPSC on hydroxyapatite/tricalcium phosphate structure grafted subcutaneously to mice, which were subjected to DPSC immunosuppression. After 8 weeks, the dental pulp stem cells transformed into odontoblasts and then proceeded to form the dentin [18,23]. In polarized light, the structure created by DPSC was characterized by the presence of a highly ordered collagen matrix forming on the surface of the hydroxyapatite/tricalcium phosphate molecules. Direct contact between the odontoblasts and the formed dentin was observed [11,18].

A 3-month observation of dentin formation with DSP expression showed that the grafted dental pulp cells differentiated into mature odontoblasts and accreted dentin, creating the pulp/dentin complex. It was pointed out that the culture of isolated DPSC without the participation of the factors responsible for the mineralization process proved that DPSC synthesis was absent, which in turns indicated that environmental factors were necessary for stem cells to differentiate.

The dental pulp of deciduous teeth is another source of mesenchymal stem cells. Similarly to DPSC, SHED cells also have the specific epitopes of mesenchymal cells on their surfaces, but their proliferative potential was significantly higher. Despite weaker histologic differentiation, SHED cells have bone tissue-specific markers on their surfaces, such as bone sialoproteins and specific dentin marker (dentin sialoprotein, DSP). SHED cells grafted in mice subjected to immunosuppression showed the ability to produce dentin; however, pulp/dentin complex regeneration was not histologically observed [18].

A unique feature of stem cells isolated from the dental pulp of deciduous teeth is their capability of *in vivo* osteoinduction and recruitment of bone-forming cells, and bone tissue formation [24- 27]. Following an appropriate stimulation, SHED cells, similarly to DPSC, are able to differentiate into fat and nerve cell lines [24].

#### **Periodontal ligament stem cells (PDLSC)**

Periodontal ligament stem cells are capable of self-regeneration, unlimited division and

differentiation, and have an immunomodulatory character. The stem cells of the periodontal membrane were first isolated from the root surface of extracted third molars, and were called periodontal ligament stem cells. Those cells can be isolated from tooth socket walls after dental extraction, as well as from the root surface of deciduous teeth, or even from inflamed periodontal tissue in patients with chronic periodontitis [28].

Experiments were carried out with grafting the cellular film obtained from the PDLSC culture. Cells obtained from third molars were cultured in dishes on a special substrate. In further stages, the cells separated from the substrate at low temperatures, thus creating a cellular film that then can be grafted. Cellular films were grafted into rats, in which the periodontal ligament and cementum had been previously removed. Ligament fibers and a cement-like acellular layer were formed [29]. Simultaneously, the peripheral nerve and the blood vessel grew into the root canal. Bone regeneration was achieved in experiments where cells from two groups: mesenchymal stem cells from the bone marrow and PDLSC were grafted on a hydroxyapatite/tricalcium phosphate structure. Eight weeks after implantation, new bone tissue was found [30].

The periodontal stem cell niche is located in close proximity to blood vessels, and the cells found therein have properties characteristic of stem cells, such as small size and long cell division cycle. Regeneration of the periodontal ligament depends on the presence of stem cells with specific markers, i.e. STRO1 and CD146. When cultured *in vitro*, those stem cells differentiate to cells with the properties of cementoblasts, cells synthesizing collagen, osteocytes and fibroblasts. PDLSC grafted into athymic rodents accrete cementum and structures similar to the periodontium [31]. Currently, it is assumed that periodontal stem cells originate from a small population of multipotent cells, or from numerous populations of progenitor cells found in the tooth area. This may potentially enable periodontal regeneration in clinical conditions.

#### **Dental follicle stem cells (DFSC)**

The dental sac is a condensation of mesenchymal tissue around the developing tooth bud. The activity of dental sac cells leads to the formation of the periodontal ligament and cementum [32]. The dental sac is a concentration of progenitor cells, cementoblasts, osteoblasts, and periodontal ligament cells of a multipotent character [33]. In an *in vitro* culture, the cells have the ability to transform into cells similar to cementoblasts and osteoblasts. The DFSC grafted into athymic mice produced fibrous tissue similar to periodontal ligament, and mineralized tissue similar to cementum [34].

#### **Epithelial odontogenic cells**

During the tooth formation phase, the dental crown is coated with epithelium, which disappears when the tooth erupts. Due to its continuous growth, the mouse incisor is used to analyze the mechanisms of epithelial function regulation. This arises from the division cycle of epithelial cells located in a structure called the apical loop. The epithelial stem cells divide asymmetrically into two cells; the first, called the sister cell, remains in the apical loop and is undifferentiated; the second moves towards the incisive margin and serves as the source of ameloblasts. In advanced stages of development, the dividing apical loop cells migrate in two directions, continuing the development of either the dental crown or the root [35,36]. At the beginning of the developmental cycle, the apical loop is found both in incisors and other teeth. In the case of molars, in advanced developmental stages the apical loop leaves the external and internal epithelium, the so-called Hertwig epithelial root sheath, which is a structure necessary for the root to achieve the appropriate length; it is later divided and replaced by cementoblast precursors, called epithelial cell rests of Malassez [37].

#### **Growing live flipper teeth**

In many research centers, studies on creating a biological flipper tooth are ongoing. Two main approaches are followed: *in vitro* proliferation and seeding of cells on a polymer structure, followed by implantation of the structure, or the implantation of appropriately selected and modified cells without seeding onto a structure. The cells to be grafted should have intrinsic odontogenic potential, or induced by growth and transcription factors [38]. Related experiments are conducted on the cells of the tooth and its bud, the periodontal structures, and the mesenchymal bone marrow cells [39]. Having been grafted into the tooth socket, the colonies of embryonal epithelial and mesenchymal cells obtained from the tooth bud and oral cavity epithelium began the odontogenic process, thus creating dental structures. The obtained structures were characterized by typical dental tissues; however, the distribution of those tissues was rather chaotic and the structures failed to acquire full dimensions and form [40,41]. The key limitation for the process of growing biological flipper teeth is the absence of an appropriate source of non-embryonal cells able to take up the epithelial functions of the tooth bud cells.

Initial studies of artificial teeth development consisted of observing the growth of tooth buds grafted in early developmental stages. As a result, fully formed dental crowns and partially formed roots were obtained. Research conducted by Glasstone was published in 1936 [42] and was followed by Slavkin in 1968 [43], Kollar in

1969, and Koch in 1972 [44]. The literature of the subject also includes reports of attempts to use cells originating from mesenchymal papilla and the enamel organ. As a result, dentin- and enamel-like structures were obtained. The next step was the attempt to generate a tooth from dental pulp cells [45].

#### **Stem cells from other tissues used in dental tissue regeneration**

Another tissue from which stem cells can be obtained is the bone marrow. The mesenchymal bone marrow stem cells (BMSC) grown together with embryonal epithelial cells of the oral cavity transform into cells similar to odontoblasts, containing the marker protein of odontogenesis. After grafting bone marrow cells under the renal capsule of adult rats, the development of structures similar to teeth surrounded by soft and bone tissue was observed [46].

Another structure with odontogenic potential is the hair follicle. With the induction by the tooth bud mesenchyme, its cells are able to differentiate into odontoblasts [47].

According to Huo et al., cells that, when stimulated with growth factors, are able to differentiate into odontoblasts are dermal multipotent cells (DMS) [48].

#### **Other applications of dental tissue stem cells**

Dental tissue stem cells are highly flexible, which, aside from regenerating the dental and periodontal structures, also allows them to differentiate into osteoblasts, chondrocytes, myocytes, adipocytes and neurons. Bone tissue obtained from dental tissue stem cells is the future of neurodegenerative disease treatment. Cells are obtained from the dental pulp of deciduous teeth, enabling the synthesis of osteocalcin, a protein characteristic for odontoblasts. After 30 days of growth, the formation of immature bone tissue was observed [49].

#### **Stem cells – perspectives and risks**

The ability to apply stem cells to the treatment of diseases has resulted in the emergence of cell banks storing cells from various tissues, e.g. bone marrow, as well as stem cells from permanent and deciduous teeth. Long-term storage of cells makes it possible to restore all biological functions inhibited by the cryopreservation process. Cryopreservation uses extremely low temperatures and protective chemical agents. The study by Woods et al. confirmed that the properties of a cryopreserved tooth do not differ from those of a normal tooth. The periodontal ligament cells retain their regenerative properties and the dental pulp can still serve as the source of DPSC. A low storage temperature does not affect the regenerative

properties of stem cells from apical papilla (SCAP) [50].

## **CONCLUSIONS**

Despite the risks listed above, stem cells remain the future of medicine and dentistry. With stem cells, dentists may be able to successfully treat periodontal diseases and caries, regenerate dental pulp, the periodontal membrane, and alveolar ridge bones. Targeted stem cells could improve the prognosis for dental replantation and transplantation. The outcomes of the studies discussed herein are promising and may lead to the ability to develop new tooth buds on toothless sites of the jaw bone.

## **Conflicts of interest**

We declare that we have no conflicts of interest.

## **REFERENCES**

1. Banaś A. Komórki macierzyste-perspektywy i zagrożenia. *Prz Med Uniw Rzesz Inst Leków.* 2010;2:117-27. (Polish)
2. Jones L. Stem cells: So what's in a niche? *Current Biol.* 2001;Jun 26;11(12):484-6.
3. Watt F., Hogan B. Out of Eden: Stem Cells and Their Niches. *Science.* 2000 Feb 25;287(5457):1427-30.
4. Fitzgerald M, Chiego D J Jr, Heys DR. Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. *Arch Oral Biol.* 1990;35(9):707-15.
5. Olender E, Kamiński A, Uhrynowska-Tyszkiewicz I, Wanyura H. Komórki macierzyste tkanek zęba i możliwości odtwarzania struktur zęba-przegląd piśmiennictwa. *Czas Stomatol.* 2010;63(11):682-92. (Polish)
6. 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 USA.* 2000 Dec 5;97(25):13625-30.
7. Arthur A, Rychkov G, Shi S, Koblar S A, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. *Stem Cells.* 2008 Jul;26(7):1787-95.
8. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. *Cell Death Differ.* 2007 Jun;14(6):1162-71.
9. Graziano A, d'Aquino R, Laino G, Papaccio G. Dental pulp stem cells: a promising tool for bone regeneration. *Stem Cell Rev.* 2008 Spring;4(1):21-6.

10. Sonoyama W, Liu Y, Yamaza T, Tuan R S, Wang S, Shi S, Huang G. Characterization of apical papilla and its residing stem cells from human immature permanent teeth-a pilot study. *J Endod.* 2008 Feb;34(2):166-71.
11. Gronthos S, Mankani M, Brahimi J, Robey P G, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA.* 2000 Dec 5;97(25):13625-30.
12. Maciejewska I. Wpływ zmian ekspresji genu TWIST1 i czynnika transkrypcyjnego E2A na różnicowanie i rozwój komórek macierzystych z ludzkich zębów mlecznych i stałych oraz zawiązków zębów myszy. *Ann Acad Med Gedanensis.* 2009;29(4):123 (Polish)
13. Liu H, Gronthos S, Shi S. Dental Pulp Stem Cells. *Methods Enzymol.* 2006;419:99-113.
14. Kadar K, Kiraly M, Porcsalmy B, Molnar B, Racz G Z, Blazsek J, Kallo K, Szabo E L, Gera I, Gerber G, Varga G. Differentiation potential of stem cells from human dental origin-promise for tissue engineering. *J Physiol Pharmacol.* 2009 Dec;60 Suppl 7:167-75.
15. Perry BC, Zhou D, Wu X, Yang FC, Byers MA, Chu TM, Hockema JJ, Woods EJ, Goebel WS. Collection. Cryopreservation and Characterization of human dental pulp derived mesenchymal stem cells for banking and clinical use, tissue engineering: *Tissue Eng Part C Methods.* 2008 Jun;14(2):149-56
16. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res.* 2003 Apr;18(4):696-704.
17. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. *J Endod.* 2008 Jun;34(6): 645-51.
18. Gronthos S, Brahimi J, Li W, Fisher LW, Cherman N, Boyde A, Denbesten P, Robey G, Shi S. Stem cells properties of human dental pulp stem cells. *J Dent Res.* 2002 Aug;81(8):531-5.
19. Goldberg M, Smith AJ. Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. *Crit Rev Oral Biol Med.* 2004 Jan 1;15(1):13-27.
20. D' Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosaa, Papaccio G. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone and tissue formation. *Cell Death Differ.* 2007 Jun;14(6): 1162-71.
21. Kuo, Lan WH, Lin SK. Collagen gene expression in human dental pulp cells cultures. *Arch Oral Biol.* 1992 Nov; 37(11):945-52.
22. Mangano C, De Rossa, Desiderio V, D' Aquino R, Piatellia, De Francesco F, Tirino V, Mangano F, Papaccio G. The osteoblastic differentiation of dental pulp stem cells and bone formation on different titanium surface textures. *Biomaterials.* 2010 May;31(13):3543-51.
23. Yen A, Sharpe PT. Stem cells and tooth tissue engineering. *Cell Tissue Res.* 2008 Jan;331(1):359-72.
24. Huang G, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissue vs. Those from other sources: their biology and role in regenerative medicine. *J Dent Res.* 2009 Sep;88(9):792-806.
25. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. Stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA.* 2003 May 13;100(10):5807-12.
26. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikui T, Akiyama K, Lee JS, Shi S. SHED repair critical-size calvarial defects in mice. *Oral Dis.* 2008 Jul;14(5):428-34.
27. Zheng Y, Liu Y, Zhang CM, Zhang HY, Li WH, Shi S, Le AD, Wang SL. Stem cells from deciduous tooth repair mandibular defect in swine. *J Dent Res.* 2009 Mar; 88(3):249-54.
28. Górski B. Komórki macierzyste więzadła ożębnowego - krok bliżej w kierunku rozwikłania tajemnicy regeneracji tkanek przyzębia, czyli co już wiemy i na jakie pytania należy jeszcze odpowiedzieć. *Postepy Biol Komorki.* 2013;40,4:659-82. (Polish)
29. Flores MG, Hasegawa M, Yamato M, Takagi R, Okano T, Ishikawa I. Cementum-periodontal ligament complex regeneration using the cells sheet technique. *J Periodontal Res.* 2008 Jun; 43(3):364-71.
30. Kim SH, Kim KH, Seo BM, Koo KT, Kim TI, Seol YJ, Ku Y, Rhyu IC, Chung CP, Lee YM. Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study. *J Periodontol.* 2009 Nov;80(11): 1815-23.
31. Seo BM, Miura M, Gronthos S, Bartold P M, Batouli S, Brahimi J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human perinatal ligament. *Lancet.* 2004; Jul 10-16;364(9429):149-55.
32. Kmieć Z. Histologia i cytofizjologia zęba i jamy ustnej. Elsevier Urban & Partner, Wrocław; 2007. 7-34p. (Polish)
33. Luan X, Ito Y, Dangaria S, Diekwisch T G. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. *Stem Cells Dev.* 2006; Aug;15(4):595-608.
34. Handa K, Saito M, Tsunoda A, Yamauchi M, Hattori S, Sato S, Toyoda M, Teranaka T, Narayanan AS. Progenitor cells from dental follicle are able to form cementum matrix in vivo. *Connect Tissue Res.* 2002;43(2-3):406-8.

35. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. Localization of putative stem cells in dental epithelium and their association with notch and FGF signaling. *J Cell Biol.* 1999 Oct 4;147(1):105-20.
36. Tummers M, Thesleff I. Root or Crown: A development choice orchestrated by the differential regulation of the epithelial stem cell niche of the tooth of two rodent species. *Development.* 2003 Mar;130(6):1049-57.
37. Harada TT, Toyoshima K, Yamasaki M, Itho N, Kato M, Sekine K, Ohuchi K. FGF10 Maintains stem cell compartment in developing mouse incisors. *Development.* 2002 Mar;129(6):1533-41.
38. Olender E, Kamiński A, Uhrynowska-Tyszkiewicz I, Wanyura H. Aspekty histologiczne i molekularne mechanizmy kontroli naturalnego zęba. *Czas Stomatol.* 2010; 63(9):543-50. (Polish)
39. Yen A, Sharpe P. Stem cells and tooth tissue engineering. *Cell Tissue Res.* 2008 Jan; 331(1):359-72.
40. Dualibi MT, Dualibi SE, Young CS, Barlett JD, Vacanti IP, Yelick PC. Bioengineered teeth from cultured rat tooth bud cells. *J Dent Res.* 2004 Jul; 83(7):523-8.
41. Dualibi SE, Dualibi MT, Zhang W, Asrican R, Vacanti IP, Yelick P. Bioengineered dental tissues grown in the rat jaw. *J Dent Res.* 2008 Aug;87(8):745-50.
42. Glasstone S. Development of Tooth Germs in vitro. *J Anat.* 1936; Jan;70(Pt 2):260-6.
43. Slavkin HC, Beirele J, Bavetta LA. Odontogenesis: Cell-cell interaction in vitro. *Nature.* 1968;217:269-70.
44. Kollar EJ, Baird G. The influence of the dental papilla on the development of tooth shape in embryonic mouse germs. *J Embriol Exp Morph.* 1969;Feb;21(1):131-48.
45. Koch WE. Tissue interaction during in vitro odontogenesis. in: Slavkin H.C, Bavetta L.A. editors *Developmental Aspects of Oral Biology*, Academi Press, New York, 1972;151-64.
46. Li ZY, Chen M, Liu L, Lin YF, Li AW, Tian WD. Odontogenic potential of bone marrow mesenchymal stem cells. *J Oral Maxillofac Surg.* 2007 Mar;65(3):494-500.
47. Wu G, Deng ZH, Fan XJ, Ma ZF, Sun YJ, Ma DD, Wu JJ, Shi JN, Jin Y. Odontogenic potential of mesenchymal cells from hair follicle dermal papilla. *Stem Cells Dev.* 2009 May;18(4):583-9.
48. Huo N, Tang L, Yang Z, Quian H, Wang Y, Han C, Gu Z, Duan Y, Jin Y. Differentiation of dermal multipotent cells into odontogenic lineage induced by embryonic and neonatal tooth germ cell-conditioned medium. *Stem Cells Dev.* 2010 Jan;19(1):93-104.
49. Wang J, Wang X, Sun Z, Yang H, Shi S, Wang S. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. *Stem Cells Dev.* 2010 Sep;19(9):1375-83.
50. Woods EJ, Perry BC, Hockema JJ, Larson L, Zhou D, Goebel WS. Optimized cryopreservation method for human dental pulp-derived stem cells and their tissues of origin for banking and clinical use. *Cryobiology.* 2009 Oct;59(2):150-7.