Stem cells are the hope of modern stomatology

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ABSTRACT

Introduction: Stem cells are often hailed as the medicine of the 21st Century. They provide with 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 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 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 degradation of periodontal tissue, or even heal the teeth.
Key words: Stem cells, stomatology

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INTRODUCTION

Stem cells are often hailed as "medicine of the 21st Century" or "the key to longevity". With the stem cells, in the near future the scientists will be able to slow down the process of aging and significantly extend human life span. Stem cells serve as a reservoir of cell with various degrees of development and differentiation, which have the ability to regenerate damaged organs and tissue. Their characteristic feature is the ability to regenerate and transform into specialized tissue and organs, thus making stem cells a promising solution for developing therapies against conditions such 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 [3]. Stem cells have also been found in dental and periodontal tissue. Their properties and potential application in various fields of stomatology are being studies. Those cells can be found in embryonal dental development stages, as well as in the pulp of mature teeth. The 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 the 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 also those cells 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, the cells from both cell lines are sampled from unerupted third molars which begin to develop around the age of 6, when DPSC and SCAP are on 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 the cells are 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 magnetic cell sorting system (MACS). Next, the cells were sorted, incubated, washed in 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, β-glycerophosphate and L-ascorbic acid [10,11,14,15]. The culture medium was replaced every three days. After the cells have reached an 80% confluence, understood as the coverage of the culture dish by 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 that the cells from analogous control and study groups after 6 weeks of growth. Thus, the proliferative potential of cells in 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]. The 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 more primary character both in standard and stimulated culture conditions.

The dental pulp stem cells (DPSC) and stem cells from human exfoliated deciduous teeth (SHED)

The stimulated tissue regeneration with the use of stem cells is a dynamic process comprising two components: implanted cells and culture environment [18]. The studies on the properties of DPSC and SHED completed thus far have confirmed that it is possible to apply the 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 dental pulp exposure site. As it turns out, the deficiencies of mineralized dental tissue are not regenerated; hence the studies on clinical application of DPSC and SHED are fully justified. Those studies are focusing 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 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 which can form a colony. Under a microscope, DPSC appear similar to fibroblasts. They have an elongated shape with centrally located nucleus and 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]. The 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 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 which 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 forming the dentin [18,23]. In polarized light, the structure created by DPSC was characterized by the presence of highly ordered collagen matrix forming on the surface of the hydroxyapatite/tricalcium phosphate molecules. A 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 on their surface the specific epitopes of mesenchymal cells, but their proliferative potential was significantly higher. Despite weaker histologic differentiation, SHED cells have on their surfaces the bone tissue-specific markers, such as bone sialoproteins and specific dentin marker (dentin sialoprotein, DSP). The SHED cells grafted in mice subjected to immunosuppression have shown the ability to produce dentin; however, the pulp/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, the SHED cells, similarly to DPSC, are able to differentiate into fat and nerve cell lines [24].

Periodontal ligament stem cells (PDLSC)

The periodontal ligament stem cells are capable of self-regeneration, unlimited division and differentiation, and have immunomodulatory character. The stem cells of the periodontal membrane were first isolated from the surface of the roots of extracted third molars, and were called

the periodontal ligament stem cells. Those cells can be isolated from tooth socket walls after dental extraction, as well as from the surface of roots 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 PDLSC culture. Cells obtained from third molars are cultured in dishes on special substrate. On further stage, the cells separate from the substrate at low temperatures, thus creating a cellular film that can be then grafted. Cellular films are grafted into rats, in which the periodontal ligament and cementum have been previously removed. Ligament fibers and a cement-like acellular layer were formed [29]. At the same time, 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. After eight weeks from implantation, new bone tissue was found [30]. The periodontal stem cells niche is located in close proximity to blood vessels, and the cells found therein have properties characteristic for 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 periodontium [31]. Currently, it is assumed that the 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 with 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 produce 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 the 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. On 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 in other teeth. In the case of molars, on advanced developmental stages the apical loop leaves an 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 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]. The 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 typically 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 in observing the growth of tooth buds grafted on 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] and 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. the cells which, when stimulated with growth factors, are able to differentiate into odontoblasts are dermal multipotent cells (DMS) [48].

Other applications of dental tissue stem cells

The 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 treatment of neurodegenerative diseases. The cells are obtained from 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 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 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. 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 the periodontal diseases and caries, regenerate dental pulp and 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 the toothless sites of the jaw bone.

Conflicts of interest

We declare that we have no conflicts of interest.

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