CPQ Dentistry (2019) 1:4
Review Article

Oral Derived Stem Cells Potential - Current Status

Artur Bryja[1], Katarzyna Stefańska[2], Joanna Budna-Tukan[2], Bartosz Kempisty[1,2,3] & Marta Dyszkiewicz-Konwińska[1,4*]

[1]*Department of Anatomy, Poznan University of Medical Science, Poznań, Poland*

[2]*Department of Histology and Embryology, Poznan University of Medical Science, Poznań, Poland*

[3]*Department of Obstetrics and Gynecology, University Hospital and Masaryk University, Czech Republic*

[4]*Department of Biomaterials and Experimental Dentistry, Poznan University of Medical Sciences, Poznań, Poland*

[*]**Correspondence to:** Dr. Marta Dyszkiewicz-Konwińska, Department of Anatomy & Department of Biomaterials and Experimental Dentistry, Poznan University of Medical Science, Poznań, Poland.

Copyright

Received: 21 May 2019

Published: 17 June 2019

Keywords: *Oral Stem Cells; Mesenchymal Stem Cells; Exosomes; Differentiation; Stem Cells Banking; Stem Cells Freezing*

Abstract

Recently the potential of oral derived stem cells is being extensively studied.

There is a significant number of research conducted on different stem cells from pulp cavity as well as from periodontal ligament, dental follicle and gingiva.

How far are we from applying them into the practice?

What is already available for clinical application. What knowledge should we possess to successfully benefit from their potential. We have analyzed numerous scientific papers in order to summarize current status of dental stem cells.

Oral Derived Stem Cell Properties

Types of Stem Cells in the Oral Cavity

Dental tissues are a potent source of stem cells, that can be used not only in regeneration of dental tissues themselves, but in other cellular and regenerative therapies as well. Stem cells from the oral cavity are common in dental tissues and easy to obtain, since in many cases the tissues they are isolated from would be discarded as a medical waste. Moreover, these cells belong to adult stem cells, thus there are no ethical concerns compared to embryonic stem cells.

All of the stem cells obtainable from oral cavity belong to mesenchymal stem cells (MSC), which means that they have to meet the minimal criteria established by The International Society for Cellular Therapy (ISCT). Such criteria include the ability to adhere to plastic in standard culture conditions, the positive expression of CD105, CD73, CD90 and lack of CD45, CD34, CD14 or CD11b, CD79alpha or CD19, HLA-DR, as well as the ability to self -renew and differentiate into osteoblasts, adipocytes and chondroblasts *in vitro* [1].

Human oral cavity-derived MSC include nine types of cells: dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs), alveolar bone-derived MSCs (ABMSCs), stem cells from apical papilla (SCAP), gingival MSCs (GMSCs), oral mucosa stem cells (OMSCs) and salivary gland-derived stem cells (SGSCs) [2,3].

Dental pulp stem cells (DPSCs) were the first type of cells isolated from human permanent teeth dental pulp, whereas stem cells from human exfoliated deciduous teeth (SHED) were obtained from dental pulp of infants' teeth. Both of these cell types have higher proliferation capacity than bone marrow mesenchymal stem cells (BMMSC), and are easily accessible and isolated. Apart from being the source of odontoblasts and the ability to differentiate into osteoblasts, adipocytes and chondroblasts, DPSCs can also develop into neurons, cardiomyocytes, myocytes, melanocytes and hepatocyte-like cells. Moreover, they have immunomodulatory properties, thus could be used as immunosuppressors during inflammatory process. Other potential clinical application of DPSCs include reconstitution of dentin/pulp complexes, neural tissues, bone, cementum and blood vessels, treatment of systemic diseases such as spinal cord injury, Parkinson's disease, Alzheimer's disease, cerebral ischemia, myocardial infarction, muscular dystrophy, diabetes. SHED have even higher proliferation rate than DPSCs and express OCT4 and NANOG, which are embryonic stem cell markers and can be utilized in aforementioned therapies as well [4,5].

Periodontal ligament originates from the dental follicle, surrounds the tooth root and belongs to connective tissues. Stem cells obtained from this tissue (periodontal ligament stem cells - PDLSCs) are able to differentiate into neural, endothelial, cardiac and Schwann cells, apart from the cell types typical for MSCs, and have higher proliferation capacity than BMMSCs. Their application in bone, tendon and neural regeneration has been demonstrated *in vivo*. Similarly to SHED, PDLSCs express OCT4 and NANOG, as well as SSEA-1, SSEA-3, SSEA-4 and SOX2. PDLSCs exhibit also immunomodulatory properties and suppress proliferation of peripheral blood mononuclear cells (PBMCs) [6].

Dental follicle surrounds developing tooth germ, and dental follicle progenitor cells (DFPCs) were isolated from human third molar teeth for the first time. These cells may differentiate into cementoblasts, periodontal ligament cells, osteoblasts, adipocytes, chondrocytes and neurons. When cultured with dexamethasone, DFPCs produce compact calcified nodules. Apart from typical markers for MSCs, the expression of NOTCH1 and NESTIN in DFPCs has been described [7]. The tissue that originates from dental follicle is alveolar bone, which is a good source of stem cells, and their collection is minimally painful for the patient. Alveolar bone-derived MSCs (ABMSCs) can be used in bone regeneration, as they have high osteogenic potential both *in vitro* and *in vivo*. ABMSCs are also able to differentiate to a lesser extent into chondrocytes and adipocytes [8].

Stem cells from the apical papilla (SCAP) have been isolated from the apical papilla of immature permanent teeth, which means that they originate from the developing tissue. They have higher proliferation capacity than DPSCs and greater migration ability. SCAP express markers typical for other MSCs, but can be distinguished from DPSCs by the presence of CD24, as well as higher expression of survivin, longer telomeres and higher telomerase activity. These cells are able to differentiate into various cell lineages, such as osteoblasts, odontoblasts, adipocytes, chondroblasts, neurons and hepatocyte-like cells. The fact that they possess low immunogenicity and inhibit T cell proliferation makes them potentially beneficial in stem cell-based therapies [9].

Another promising source of stem cells from oral cavity are gingival tissue and oral mucosa, as they are often discarded during dental procedures and their collection is minimally invasive for the patient. Gingiva is a part of biological mucosal barrier and surrounds the teeth in their sockets in the alveolar bone. It is known for its wound healing properties and gingival MSCs (GMSCs) exert anti-inflammatory effects, inhibit T cell proliferation and have unique immunomodulatory function, which suggests that it would be beneficial to utilize them in inflammatory diseases therapies. Moreover, GMSCs exhibit osteoblastic, adipocytic, chondrocytic, endothelial and neural differentiation potential, thus can be used in these tissues regeneration [10,11]. Oral mucosa stem cells (OMSCs) share many similarities with GMSCs, however they originate from the subepithelial layers of oral mucosa. Both of these cell types have high proliferation rate and express markers typical for MSCs [12].

Stem cells have also been isolated from human salivary glands (salivary gland-derived stem cells SGSCs). These cells are able to self-renew and differentiate into functionally adult tissue, which was confirmed by the expression of cytokeratin, α-amylase and AQP-5. After the transplantation into the irradiated salivary glands, SGSCs restored saliva production, which makes them a promising therapeutic agent in xerostomia treatment [13].

Exosomes

Extracellular vesicles (EVs) have become of great interest due to their special role in intercellular communication. When we take into account the size of the vesicles and their functions, we can divide them into three groups (1) apoptotic bodies - 800-5000nm, they are secreted during apoptosis; (2) ectosomes - 50-1000nm, they are assembled and released from the plasma membrane; (3) exosomes - 40-100nm, they are released during exocytosis [14].

Exosomes are secreted by normal and pathological cells [15]. Extracellular vesicles and exosomes are created by proteins and lipids. Inside them, bioactive molecules such as DNA, RNA, miRNA, peptides, and proteins can be transported [15]. Exosomes participate in intercellular communication and control of biological processes. Molecules inside exosomes can be transported to other cells. The molecules inside the exosomes are also protected from degradation. An example of this is RNA transport inside exosomes. RNA can be transported from one cell to another cell in which it becomes a matrix for protein synthesis. Vesicles secreted by stem cells stimulate proliferation and inhibit the apoptosis [15].

Exosomes can be isolated from *in vitro* culture. For isolation commercial isolation kit may be used or serial centrifugation. Such commercial kit was used by Rager *et al.* They cultured bone marrow-derived mesenchymal stem cells (BM-MSCs) in the culture flask. When cells have 80% confluent, the culture medium was changed to serum-free medium for 48h. After this time from conditioned medium (CM) were isolated exosomes using two methods. In the first method was used the commercial kit for exosomes isolation (P100 PureExo Exosome Isolation Kit) [16]. In the second method, cells were collected and centrifuged at 300g for 10min. Next, cells were removed. The supernatant was collected and centrifuged at 2000g for 20min. The resulting supernatant was again centrifuged at 10000g for 30min to the pellet of cellular debris, which was discarded. In the last step, the supernatant was centrifuged at 100000g for 18h to obtain isolated exosomes (pellet) and exosome-depleted conditioned medium (supernatant) [16]. It is generally proposed that centrifugation for 18h be used to eliminate EVs provided with FBS. Shelke *et al.* showed that EVs from FBS could substantially influence cultured cell behaviour. They tested different times of centrifugation and concluded that ultracentrifugation for 18h removed 95% of FBS EVs in the cell culture medium [17].

The stem cell exosomes are heterogeneous. They contain different information depending on the type of stem cell. In regenerative medicine it is important to find the right stem cells, that will secrete exosomes, that stimulate angiogenesis. These exosomes stimulate tissue replacement and ensure the proper development of the newly formed tissue [15].

Potential of Stem Cells in General Medicine

Stem cells are primitive and immature types of cells with an ability to self-renew and differentiate into mature cell types, which makes them an attractive tool for regenerative medicine and treatment of various diseases. Stem cells obtained from embryos (embryonic stem cells - ESC) are totipotent (blastomeres) or pluripotent (when collected at the later stage of development), which means that they can differentiate into all of the cell types in human organism. However, to obtain these cells, an embryo must be destroyed and the use of ESC is burdened with the risk of formation of teratomas. Utilizing adult stem cells (ASCs) allows to avoid such threats and ethical concerns, as ASCs are present in adult and fetal tissues such as bone marrow, adipose tissue, liver, umbilical cord blood, and are involved in damaged tissue regeneration, although they have lower developmental potential than ESC. Amongst ASCs, the mesenchymal stem cells (MSCs) seem to be the most promising in diseases treatment, as they are easily obtainable and have high proliferation rate and developmental capacity [18].

MSCs are known for their immunomodulatory properties and can exert both immune suppressor and enhancer functions. They are also able to establish functional cross-talk with immune mediators and express specific receptors for chemokines and cytokines. Such features make MSCs a promising tool in inflammatory

diseases treatment, such as asthma, inflammatory bowel disorders or skeletal disorders, as well as the graft versus host disease [19]. Another potential application of stem cells is cardiovascular diseases treatment, especially cardiac muscle regeneration. Cardiovascular diseases are one of the leading causes of morbidity and mortality in the world and their result is often an irreversible loss of cardiac tissue. Several stem cell types have already been utilized in animal models and clinical trials, including skeletal myoblasts, bone marrow MSCs and HSCs, cardiac progenitors, adipose-derived stem cells (ASCs), induced pluripotent stem cells (iPSCs) and ESCs. Such therapies may improve the damaged tissue regeneration through paracrine mechanisms and cardiac differentiation of stem cells; however this method requires further optimization [20].

Neurological disorders that are caused by the loss of neurons and glial cells, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, ALS, multiple sclerosis, stroke and spinal cord injury, are another promising therapeutic target of stem cell-based therapies. Stem cells may act via production of neuroprotective growth factors and therefore support neural restoration, or differentiate into neurons or glial cells. However, it is still uncertain which source of stem cells would be the most beneficial, as the active neurons have been obtained from ESCs, MSCs and neural stem cells (NSCs), thus further studies have to be carried out [21].

Stem cells may also be useful in musculoskeletal system regeneration, especially for treatment of bone defects, osteonecrosis, focal osteochondral defect, osteoarthritis and tendon regeneration, since cell injection would be less invasive than surgical procedures. The bone marrow and the adipose tissue are the most common sources of stem cells for orthopedic therapies, however, despite the abundance of basic and pre-clinical studies, not much reliable clinical data have been published [22].

Other potential applications of stem cell-based therapies include treatment of several diseases, such as cancer (since many ASCs exhibit tumor-tropic properties [23], kidney disease [24], degenerative eye disease [25], lung diseases [26] and many other, that are beyond the scope of this paper.

Potential of Stem Cells in Dentistry

With the current knowledge the most interesting area of stem cells in dental therapies is application of exosomes as a carrier or stimulator in pulp regeneration or periodontal therapies. Without extended manipulation these microvesicles have an anti-inflammatory effect and could be applied into the clinical practice in a similar manner as PRF.

Dental stem cells are also known to produce mineralized matrix and are easily differentiated into osteoblasts. Together with biomaterials or individualized matrices can successfully stimulate bone growth.

With the growing number of cases developing periimplantitis the potential of dental stem cells is becoming even more interesting. Therapy involving patients' own exosomes or stored and multiplied SC could become essential part of the treatment. In maxillofacial surgery in the need of major reconstruction dental stem cells together with designed scaffolds could significantly benefit the outcome of treatment. They are also tested for potential application in periodontal diseases. Autologous PDLSCs have shown promising results in preclinical studies. Availability and function of PDLSCs is influenced by the age and health status of donors [27]. The proliferative capacity, migratory potential, diminishes with progressing age.

That is the reason why banking of material from younger individuals again seems reasonable.

Potential of Stem Banking

Method of Deriving Stem Cells

As it is widely known there are many sources of stem cells in the human body. The ones of hematopoietic origin are found in the bone marrow and its isolation requires anesthesia and surgical intervention. Aspiration of adipose tissue, which is a rich source of mesenchymal stem cells, although simpler is still a complex procedure. Discovery of dental stem cells opened a broad range of possibilities in the field of low-invasive stem cells recovery and storing [28]. Among dental tissues, like dental papilla, periodontal ligament and gingiva, the most prominent source of dental stem cells is a dental pulp [29]. The tissue is rather easily accessible and relatively simple to harvest [30]. Additionally, although some differences in proliferation potential are observed, DPSCs can be collected from both - deciduous teeth and adult teeth. In the last case which make selection of the sample for dental stem cell banking has to comply strict criteria. As far as deciduous teeth are concerned, anterior are preferred over posterior, as well as extracted over exfoliated. They should present vascularized pulp and the two third of the preserved root. Respectively, adult teeth should be vital, infection-free, without any signs of periodontal diseases and possessing satisfying amount of pulp [31,32].

The whole process of DPSCs harvesting can be divided into two parts - extraction in the dental office and post-extractional laboratory protocol. Firstly, the dental surgeon, after obtaining written consent of the patient or parents in case of the minor, extracts the tooth in the sterile environment with the special care of preserving intact crown. After the procedure the tooth is immediately disinfected in 70% ethanol, washed in phosphate buffered saline supplemented with antibiotics and placed in the vial containing preserving medium for the time of shipment. In the next step, after arrival to the laboratory, the tooth is processed. The intact tooth can be directly stored in the liquid nitrogen, however dental pulp tissue or dental pulp cells storage is the most common. Thus, the pulp is retrieved either by sectioning the tooth with cooled diamond disc, preventing the pulp from overheating, or by sterile barbed broach. Isolated pulp is then minced and/or enzymatically digested in order to obtain single-cell suspension. Cells can be then cultured and before cryopreservation evaluated for viability, biological safety, proliferation potential and respective stem cell markers [33].

Transportation

Transport of the sample from the dental office to the laboratory is an essential step. Dental pulp, apart from stem cells, contains also abundant extracellular matrix, composed of collagen fibers and ground substance. Previously it was thought that these tissues cannot be efficiently frozen [34]. Nowadays we know that it is possible, however special care has to be taken to protect them during shipment [35]. Transportation kit must provide optimal conditions for the tissue to keep it vital and nourished. The vials destined for the tissue should be filled with approx. 20mL of previously chilled transport solution. Currently there are few commercially available agents designed for this purpose, however studies showed that phosphate buffered saline supplemented with antibiotics is of comparable quality [36]. Transport should be carried on ice or in

vacuum containers, maintaining required temperature of 2-8°C. Studies on animals revealed that the time between tooth extraction and cryopreservation is essential for viability of cells post-thawing [37]. Lin *et al.* mechanically and histologically examined the influence of the transportation time prior to storage and concluded that period of 24h, followed by programmed-freezing does not impair the quality of the cells [34]. In accordance to this observation, stem cell banks worldwide prefer overnight shipping that takes no longer than 24h [38]. However, some authors describe even longer - 48h period of shipment under room temperature [33]. Perry *et al.* tested the time frame in which the extracted teeth can be processed based on the ability of DPSC to establish cultures. Interestingly, they proved that samples stored in 4°C after arrival to the laboratory can be processed not only within 48h but even up to 120h post-extraction, what makes banking even more feasible [36].

Testing

In the case of stem cell banking, tests are carried out to determine cell viability, and microbiological tests are carried out for the presence of viruses, bacteria, and fungi. The currently used tests in cell cultures use the PCR technique [39,40]. The development of the PCR method was a breakthrough for clinical diagnostics as well as for the control of cell cultures [41].

The standard method used for testing cells viability is trypan blue exclusion test. For this analysis, 10 ml of the cell suspension are taken. To cell suspension was added 10 ml of 0.4% Trypan Blue Solution. Cells are counted using hemocytometer. The viable cells are clear and non-viable cells are blue [42]. In recent years, automated instrumentation has been used to check cell count and cell viability [43].

Freezing

Studies conducted on DPSCs after thawing enabled to establish the protocol of most effective long-term preservation. The post-thaw recovery and reproducibility were of primary importance. The procedure of cells evaluation was based on the growth rate, expression of mesenchymal markers, including CD73, CD90, CD105, and lack of CD34, CD45, CD11b, CD19, HLA-DR markers. Additionally their multilineage differentiation capacity was assessed.

Although no differences were found in post-thaw viability and multilineage differentiation capacity of cells frozen in -85°C and -196°C for 6 months [38], the most common way of long-term storage is cryopreservation in the liquid nitrogen (LN2). Comparison of rapid freezing and controlled-rate freezing showed significant prevalence of the latter in the aspect of cells viability. It can be assured by the use of controlled-rate freezing chambers, which reduce the temperature at the rate of -1°C per minute while placed in -80°C for 24h before samples allocation in the LN2 [44] or protocols based on equilibration, progressive cooling to low sub-zero temperatures with the use of magnetic programmed freezer and subsequent transportation to LN2 [34, 45-47].

Among tested cryoprotective agents (CPA), like ethylene glycol, propylene glycol and dimethyl sulfoxide (Me2SO), the latter showed the best protective abilities. Cells viability post-thaw was the highest when its concentration ranged 1.0M-1.5M, which corresponded to 10% v/v solution. Described conditions were suitable for freezing of 0.5 - 2.0 x 10^6 cells per vial. However, keeping in mind potential therapeutic

of those cells, usage of non-animal-derived agents is highly recommended. Commercially available media present comparable effectiveness and are suitable for clinical banking [38].

Nowadays three options of DPSCs storage are available. The first one refers to primarily-cultured DPSCs banking. After pulp tissue digestion, obtained separated cells are cultured up to 4th passage and when achieving appropriate confluency frozen or frozen immediately after digestion. Alternative approach is based on spontaneous outgrowth of stem cells from minced pulp tissue. Experiments showed that viability of frozen and thawed after 1 month cells was comparable to freshly-cultured samples, however their growth was significantly impaired [48]. The second possibility is cryopreservation of undigested dental pulp with Me2SO protocol described previously. After thawing and plating cells were able to proliferate, manifested by the presence of at least one colony forming unit. There was no statistically significant difference between growth rate and morphology between frozen and freshly-cultured tissue. Most importantly, proliferating cells preserved their differentiation abilities towards osteoblasts, chondrocytes and adipocytes. The less interfering option seems to be cryopreservation of the intact teeth. This approach however is characterized by inconsistent outcomes. Woods *et al.* described that from 10 teeth tested, cells isolated from only three teeth presented growth typical for freshly-cultured DPSCs and could be subsequently evaluated. The rest of the teeth-isolated cells either did not attach to the culture dish or exhibited impaired growth [38]. Similarly, Lindemann *et al.*, despite typical mesenchymal phenotype and differentiation capacity, observed reduced culture and proliferation rates as well as morphological changes in DPSC derived from the frozen intact teeth comparing to control group of immediately isolated DPSCs [49]. On the other hand Perry *et al.* found that seven out of ten frozen teeth contained post-thaw viable cells, without diminished growth in culture, changes in markers expression or impaired differentiation potential [36]. This stayed in agreement with studies of Lee *et al.*, showing 73% growth rate, appropriate surface markers and differentiation ability of DPSC isolated from cryopreserved teeth [50]. The most optimistic observation was published by Gioventu *et al.*, who pierced the teeth just after extraction with an Nd:YAG laser to form the micro channels in the enamel and dentin without destroying the pulp. These pores were meant to provide better pulp infiltration with CPA added in the following step of the procedure. Results were very encouraging showing DPSCs isolated from pierced and frozen teeth of proper morphology, phenotype and growth rate comparable to DPSCs harvested from fresh teeth [51].

Based on presented data it can be concluded that cryopreservation of the intact tooth is not stable and repeatable approach for the DPSCs clinical banking is required. The recommendations for minimal manipulation and on the other hand the need to obtain the maximal growth rate, points to the compromise between whole teeth storage and storage of previously isolated and long-term cultured DPSCs. Pre-freezing pulp isolation allows its better penetration of CPA comparing to the intact tooth, where thick dentin forms an obstacle. Thus, freezing of the undigested dental pulp tissue or initial cell suspension after pulp digestion seems to be the best option. As far as freezing without digestion is concerned, post-thaw cells may be already compromised to some extent by ice formation and thus need only gentle digestion, resulting in better growth rate. On the contrary, digestion before freezing can impair cellular membrane making the cell more susceptible to cryopreservation. Above all, minimal manipulation of tissue, apart from DPSCs potential preservation, is cost effective and favors long-term banking [38].

Stem Cells Differentiation

Stem cells are characterized by their ability to self-renew and differentiate into other cells. These features of stem cells are regulated by various factors. And the knowledge of these factors is not enough. The same factor can both stimulate and inhibit stem cells differentiation [10]. The behavior of stem cells is influenced by signaling pathway such as Notch [52], Wnt [52,53], Nanog [52]. Activation of the Notch pathway influences the inhibition of differentiation processes [52]. Similarly, activation of the Wnt pathway affects the ability of stem cells to self-renew and limits their differentiation [53]. Nanog protein is active in embryonic stem cells and determines their pluripotency [54]. In self-work, Augello *et al.* also described the role of Wnt and TGFB superfamily signaling pathways in MSCs differentiation [55].

Mesenchymal Stem Cells (MSCs) are characterized by the presence CD73, CD90 and CD105 antigens on their surface. They don't show expression of CD45, CD34 and CD14 antigens [2]. Dental Pulp Mesenchymal Stem Cells (DP-MSCs) don't have specific markers. They showed expression of markers characteristic for mesenchymal and bone-marrow stem cells (STRO-1, CD146) and markers characteristic for embryonic stem cells (OCT4). The candidate markers of DP-MSCs include also CD73, CD90, CD105, CD29, CD44, CD146, CD166 and CD271 [56].

MSCs can be differentiated to osteoblasts, chondrocytes, and adipocytes. Osteogenic differentiation is induced by adding to the culture medium ascorbic acid and dexamethasone. The stimulation was provided for 2 weeks. To increase the mineralization to the medium was added β-glycerophosphate [57]. The culture may also be supplemented with calcium to increase *in vitro* mineralization [58]. The ongoing osteogenesis process can be evaluated by an enzymatic method by measuring the activity of bone-specific Alkaline Phosphatase (ALP) [58]. Another method to evaluate the osteogenic differentiation is the detection of calcium-rich deposits in cell culture with use to staining Alizarian Red S (ARS) [59]. Chondrogenic differentiation in Muraglia *et al.* study was induced by adding to the culture medium ascorbic acid and human recombinant TGFB1 for 1 week [57]. Further additives to the medium include sodium pyruvate, proline, and L-glutamine [58]. During chondrogenic differentiation, it is possible to measure the expression of chondrogenic markers such as collagen II, collagen XI, aggrecan, perlecan, and syndecan [58]. In this case, for adipogenic differentiation dexamethasone and insulin was used, for 3 weeks [57]. The effectiveness of differentiation can be assessed by using staining with oil red O or glycerol-3-phosphate dehydrogenase as a marker of the mature adipocytes [58]. Another method is to use flow cytometry in a MSC culture stained with the Nile red [58,60].

Commercially available are also ready-to-use media for the differentiation of stem cells to osteoblasts [61,62], chondrocytes [61-63], adipocytes [61,64] and neural cells [65].

The stem cells from the oral cavity may play an important role in modern dentistry. It was showed that DP-MSCs are similar to MSCs delivered from adipose tissue, bone marrow, and umbilical cord tissue. They can differentiate into osteoblasts, chondrocytes, and adipocytes [66]. Lee *et al.* showed that DP-MSCs and periodontal ligament stem cells (PDLSCs) have neurogenic differentiation potential [65]. Lee *et al.* also studied the effect of Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor-2 (FGF-2) on the PDLSCs. They showed that VEGF positive influence on osteogenic differentiation. In contrast, FGF-2 might play a role in the progenitor cells proliferation and might inhibit terminal differentiation [50]. In dental surgery, the source of the stem cells may also be orofacial. It was showed that stem cells from

orofacial have a better osteogenic differentiation potential as stem cells from the iliac crest [67].

Guidelines

DPSCs are currently tested in numerous therapeutic applications. That is why all actions involving them have to follow Food and Drug Administration's (FDA) current good tissue practice (cGTP) addressed to all establishments manufacturing human cell, tissue and cellular and tissue-based products (HCT/Ps). The superior aim of the regulations is protection of the public health by providing safe products, without introduction, transmission and spread of diseases. Thus, requirements prevent transmission of bacteria, viruses, fungi and parasites by HCT/Ps and their contamination during processing. Establishments banking stem cells have to follow regulations related to facilities, equipment, supplies and reagents, recovery, shipment, storage, donor eligibility with their screening and testing, as well as environmental, processing and labeling controls [68].

For HCT/Ps which contain or consist of human cells or tissues, are intended for implantation, transplantation, infusion or transfer into human recipient "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use" guidance applies. There are three main criteria HCT/Ps without required premarket approval should comply. Firstly, the product should be minimally manipulated. For structural tissue this means that "processing does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement" and for cells or nonstructural tissues that "processing does not alter the relevant biological characteristics of cells or tissues". Secondly, HCT/Ps should be intended for homologous use only, which means "the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor". Lastly, cells or tissues should not be combined with other articles, apart from water, crystalloids, preserving and sterilizing agents, on condition that their addition does not undermine HCT/P clinical safety (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/regulatory-considerations-human-cells-tissues-and-cellular-and-tissue-based-products-minimal).

Discussion and Conclusion

Numerous studies have shown the formation of dental tissues in animal models. A recent clinical trial in humans demonstrated the potential of adult dental mesenchymal stem cells attached on collagen to regenerate bone of the mandible. Yet we are still awaiting big breakthrough in the field of stem cells' application. How to better control the faith of stem cells in *in vivo* conditions. The methods of tracing stem cells are rapidly evolving. The future SC research should focus on basic understanding of growth regulators in differentiation and trans-differentiation and site-specific homing . To reduce the risk of oncogenic transformation special attention should be paid to the genetic safety of cell preparation.

To overcome limitations involving stem cell transplantation, exosomes' clinical potential is extensively studied. Because of their physiochemical stability in the body stem cell-derived exosomes offer a method to provide cell-free regenerative medicine. Exosomes can be easily produced in the laboratory setting and are easily identifiable due to several markers. The studies of exosmes are carried out in many leading scientific centers.

Some of the regenerative applications are already slowly introduced into the practice- it has started years ago with PRP and PRF technique. And is continued with CGF and other innovative approaches involving patients' own stem cells for example together with bioscaffolds or biomaterials.

Banking dental stem cells is already available in number of countries (StemSave, BioEden, Store-a-tooth, DentCell, Stemade, Teeth Bank Co.Ltd., Cellivia Bank) and the legislation is trying to keep up with the evolving methods. DPSC-based therapy is now entering into a new stage shifting from *in vitro* and *in vivo* studies to optimization and standarization of production processs for successful transfer into the clinical applications.

Acknowledgment:

This work was supported by IASlab Institute of Advanced Sciences in Poznań, Poland.

Bibliography

1. Dominici, M., Le Blanc, K., Mueller, I., *et al.* (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy, 8*(4), 315-317.

2. Brożek, R., Kurpisz, M. & Koczorowski, R. (2017). The oral cavity - potential source of stem cells: Jama ustna jako potencjalne źródło komórek macierzystych. *Postepy Hig Med Dosw., 71*(0), 881-894.

3. Liu, J., Yu, F., Sun, Y., *et al.* (2015). Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. *Stem Cells, 33*(3), 627-638.

4. Ledesma-Martinez, E., Mendoza-Nunez, V. M. & Santiago-Osorio, E. (2016). Mesenchymal Stem Cells Derived from Dental Pulp: A Review. *Stem Cells International, 2016*(4709572).

5. Yamada, Y., Nakamura-Yamada, S., Kusano, K. & Baba, S. (2019). Clinical Potential and Current Progress of Dental Pulp Stem Cells for Various Systemic Diseases in Regenerative Medicine: A Concise Review. *Int J Mol Sci., 20*(5).

6. Tomokiyo, A., Yoshida, S., Hamano, S., Hasegawa, D., Sugii, H. & Maeda, H. (2018). Detection, Characterization, and Clinical Application of Mesenchymal Stem Cells in Periodontal Ligament Tissue. *Stem Cells International, 2018*(5450768).

7. Morsczeck, C., Gotz, W., Schierholz, J., *et al.* (2005). Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol., 24*(2), 155-165.

8. Matsubara, T., Suardita, K., Ishii, M., *et al.* (2005). Alveolar bone marrow as a cell source for regenerative medicine: differences between alveolar and iliac bone marrow stromal cells. *J Bone Miner Res., 20*(3), 399-409.

9. Kang, J., Fan, W., Deng, Q., He, H. & Huang, F. (2019). Stem Cells from the Apical Papilla: A Promising Source for Stem Cell-Based Therapy. *Biomed Res Int., 2019*(6104738).

10. Venkatesh, D., Kumar, K. P. M. & Alur, J. B. (2017). Gingival mesenchymal stem cells. *J Oral Maxillofac Pathol., 21*(2), 296-268.

11. Zhang, Q., Shi, S., Liu, Y., *et al.* (2009). Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol., 183*(12), 7787-7798.

12. Zhang, Q. Z., Nguyen, A. L., Yu, W. H. & Le, A. D. (2012). Human oral mucosa and gingiva: a unique reservoir for mesenchymal stem cells. *J Dent Res., 91*(11), 1011-1018.

13. Pringle, S., Maimets, M., van der Zwaag, M., *et al.* (2016). Human Salivary Gland Stem Cells Functionally Restore Radiation Damaged Salivary Glands. *Stem Cells., 34*(3), 640-652.

14. Gatkowska, J. & Długońska, H. (2016). The role of extracellular vesicles in parasite-host interaction: Rola zewnątrzkomórkowych pęcherzyków błonowych w interakcji pasożyt-żywiciel. *Postepy Hig Med Dosw., 70,* 951-958.

15. Zimta, A. A., Baru, O., Badea, M., Buduru, S. D. & Berindan-Neagoe, I. (2019). The Role of Angiogenesis and Pro-Angiogenic Exosomes in Regenerative Dentistry. *Int J Mol Sci., 20*(2), 406.

16. Rager, T. M., Olson, J. K., Zhou, Y., Wang, Y. & Besner, G. E. (2016). Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotizing enterocolitis. *J Pediatr Surg., 51*(6), 942-947.

17. Shelke, G. V., Lässer, C., Gho, Y. S. & Lötvall, J. (2014). Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. *J Extracell Vesicles., 3*.

18. Szustka, A. & Rogalinska, M. (2017). Potential application for stem cells in regenerative medicine and transplantology. *Postepy Biochem., 63*(2), 143-150.

19. Rameshwar, P., Moore, C. A., Shah, N. N. & Smith, C. P. (2018). An Update on the Therapeutic Potential of Stem Cells. *Methods Mol Biol., 1842,* 3-27.

20. Muller, P., Lemcke, H. & David, R. (2018). Stem Cell Therapy in Heart Diseases - Cell Types, Mechanisms and Improvement Strategies. *Cell Physiol Biochem., 48*(6), 2607-2655.

21. Kim, S. U. & Vellis, J. de (2009). Stem cell-based cell therapy in neurological diseases: a review. *J Neurosci Res., 87*(10), 2183-2200.

22. Im, G. I. (2017). Clinical use of stem cells in orthopaedics. *Eur Cell Mater., 33,* 183-196.

23. Stuckey, D. W. & Shah, K. (2014). Stem cell-based therapies for cancer treatment: separating hope from hype. *Nat Rev Cancer., 14*(10), 683-691.

24. Lazzeri, E., Romagnani, P. & Lasagni, L. (2015). Stem cell therapy for kidney disease. *Expert Opin Biol Ther., 15*(10), 1455-1468.

25. Mead, B., Berry, M., Logan, A., Scott, R. A. H., Leadbeater, W. & Scheven, B. A. (2015). Stem cell treatment of degenerative eye disease. *Stem Cell Res., 14*(3), 243-257.

26. Esendagli, D. & Gunel-Ozcan, A. (2017). From Stem Cell Biology to The Treatment of Lung Diseases. *Curr Stem Cell Res Ther., 12*(6), 493-505.

27. Mrozik, K. M., Wada, N., Marino, V., *et al.* (2013). Regeneration of periodontal tissues using allogeneic periodontal ligament stem cells in an ovine model. *Regen Med., 8*(6), 711-723.

28. Fortier, L. A. (2005). Stem cells: classifications, controversies, and clinical applications. *Vet Surg., 34*(5), 415-423.

29. d'Aquino, R., Rosa A. de, Lanza. V., *et al.* (2009). Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater., 18,* 75-83.

30. Jo, Y. Y., Lee, H. J., Kook, S. Y., *et al.* (2007). Isolation and characterization of postnatal stem cells from human dental tissues. *Tissue Eng., 13*(4), 767-773.

31. Miura, M., Gronthos, S., Zhao, M., *et al.* (2003). SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci., 100*(10), 5807-5812.

32. Gronthos, S., Mankani, M., Brahim, J., Robey, P. G. & Shi, S. (2000). Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci., 97*(25), 13625-13630.

33. Sunil, P. M., Manikandan, R., Muthumurugan, Yoithapprabhunath, T. R. & Sivakumar, M. (2015). Harvesting dental stem cells - Overview. *J Pharm Bioallied Sci., 7*(Suppl 2), S384-6.

34. Lin, S. L., Lee, S. Y., Lin, Y. C., Huang, Y. H., Yang, J. C. & Huang, H. M. (2014). Evaluation of mechanical and histological properties of cryopreserved human premolars under short-term preservation: A preliminary study. *Journal of Dental Sciences, 9*(3), 244-248.

35. Laureys, W., Beele, H., Cornelissen, R. & Dermaut, L. (2001). Revascularization after cryopreservation and autotransplantation of immature and mature apicoectomized teeth. *Am J Orthod Dentofacial Orthop., 119*(4), 346-352.

36. Perry, B. C., Zhou, D., Wu, X., *et al.* (2008). Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. *Tissue Eng Part C Methods., 14*(2), 149-156.

37. Huang, M. S., Chang, W. J., Huang, H. M., *et al.* (2011). Effects of transportation time after extraction on the magnetic cryopreservation of pulp cells of rat dental pulp. *Journal of Dental Sciences, 6*(1), 48-52.

38. Woods, E. J., Perry, B. C., Hockema, J. J., Larson, L., Zhou, D. & Goebel, W. S. (2009). Optimized cryopreservation method for human dental pulp-derived stem cells and their tissues of origin for banking and clinical use. *Cryobiology, 59*(2), 150-157.

39. Barghouthi, S. A. (2011). A Universal Method for the Identification of Bacteria Based on General PCR Primers. *Indian J Microbiol., 51*(4), 430-444.

40. Huang, R., Zhang, J., Yang, X. F. & Gregory, R. L. (2015). PCR-Based Multiple Species Cell Counting for *In Vitro* Mixed Culture. *PLoS One., 10*(5), e0126628.

41. Morozova, A. V., Borchsenius, S. N., Vishnyakov, I. E. & Malinin, A. Y. (2017). Testing the purity of cell cultures using clinical diagnostic PCR kits. *Cell and Tissue Biology, 11*(3), 250-259.

42. Katsares, V., Petsa, A., Felesakis, A., *et al.* (2009). A Rapid and Accurate Method for the Stem Cell Viability Evaluation: The Case of the Thawed Umbilical Cord Blood. *Lab Med., 40*(9), 557-560.

43. Louis, K. S. & Siegel, A. C. (2011). Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods. In: Stoddart MJ, editor. *Mammalian Cell Viability: Methods and Protocols.* New York: Springer, 7-12.

44. Huynh, N. C. N., Le, S. H., Doan, V. N., Ngo, L. T. Q. & Le Tran, H. B. (2017). Simplified conditions for storing and cryopreservation of dental pulp stem cells. *Arch Oral Biol., 84,* 74-81.

45. Han, Y. J., Kang, Y. H., Shivakumar, S. B., *et al.* (2017). Stem Cells from Cryopreserved Human Dental Pulp Tissues Sequentially Differentiate into Definitive Endoderm and Hepatocyte-Like Cells *in vitro*. *Int J Med Sci., 14*(13), 1418-1429.

46. Shivakumar, S. B., Bharti, D., Subbarao, R. B., *et al.* (2016). DMSO- and Serum-Free Cryopreservation of Wharton's Jelly Tissue Isolated From Human Umbilical Cord. *J Cell Biochem., 117*(10), 2397-2412.

47. Park, B. W., Jang, S. J., Byun, J. H., *et al.* (2017). Cryopreservation of human dental follicle tissue for use as a resource of autologous mesenchymal stem cells. *J Tissue Eng Regen Med., 11*(2), 489-500.

48. Pilbauerova, N. & Suchanek, J. (2018). Cryopreservation of Dental Stem Cells. *Acta Medica (Hradec Kralove)., 61*(1), 1-7.

49. Lindemann, D., Werle, S. B., Steffens, D., Garcia-Godoy, F., Pranke, P. & Casagrande, L. (2014). Effects of cryopreservation on the characteristics of dental pulp stem cells of intact deciduous teeth. *Arch Oral Biol., 59*(9), 970-976.

50. Lee, S. Y., Chiang, P. C., Tsai, Y. H., *et al.* (2010). Effects of cryopreservation of intact teeth on the isolated dental pulp stem cells. *J Endod., 36*(8), 1336-1340.

51. Gioventu, S., Andriolo, G., Bonino, F., *et al.* (2012). A novel method for banking dental pulp stem cells. *Transfus Apher Sci., 47*(2), 199-206.

52. Molofsky, A. V., Pardal, R. & Morrison, S. J. (2004). Diverse mechanisms regulate stem cell self-renewal. *Curr Opin Cell Biol., 16*(6), 700-707.

53. Kleber, M. & Sommer, L. (2004). Wnt signaling and the regulation of stem cell function. *Curr Opin Cell Biol., 16*(6), 681-687.

54. Darr, H., Mayshar, Y. & Benvenisty, N. (2006). Overexpression of NANOG in human ES cells enables feeder-free growth while inducing primitive ectoderm features. *Development, 133*(6), 1193-1201.

55. Augello, A. & Bari C. de (2010). The regulation of differentiation in mesenchymal stem cells. *Hum Gene Ther., 21*(10), 1226-1238.

56. Chalisserry, E. P., Nam, S. Y., Park, S. H. & Anil, S. (2017). Therapeutic potential of dental stem cells. *J Tissue Eng., 8*.

57. Muraglia, A., Cancedda, R. & Quarto, R. (2000). Clonal mesenchymal progenitors from human bone marrow differentiate *in vitro* according to a hierarchical model. *J Cell Sci., 113*(Pt 7), 1161-1166.

58. Jackson, L., Jones, Scotting, P. & Sottile, V. (2007). Adult mesenchymal stem cells: Differentiation potential and therapeutic applications. *J Postgrad Med., 53*(2), 121.

59. Di Benedetto, A., Carbone, C. & Mori, G. (2014). Dental pulp stem cells isolation and osteogenic differentiation: a good promise for tissue engineering. *Methods Mol Biol., 1210,* 117-130.

60. Gimble, J. M., Morgan, C., Kelly, K., *et al.* (1995). Bone morphogenetic proteins inhibit adipocyte differentiation by bone marrow stromal cells. *J Cell Biochem., 58*(3), 393-402.

61. Fawzy El-Sayed, K., Graetz, C., Köhnlein, T., Mekhemar, M. & Dörfer, C. (2018). Effect of total sonicated Aggregatibacter actinomycetemcomitans fragments on gingival stem/progenitor cells. *Med Oral Patol Oral Cir Bucal., 23*(5), e569-78.

62. Herz, J., Köster, C., Reinboth, B. S., *et al.* (2018). Interaction between hypothermia and delayed mesenchymal stem cell therapy in neonatal hypoxic-ischemic brain injury. *Brain, Behavior, and Immunity, 70,* 118-130.

63. Rao, S. R., Subbarayan, R., Dinesh, M. G., Arumugam, G. & Raja, S. T. K. (2016). Differentiation of human gingival mesenchymal stem cells into neuronal lineages in 3D bioconjugated injectable protein hydrogel construct for the management of neuronal disorder. *Experimental & Molecular Medicine, 48*(2), e209.

64. Tanaka, Y., Shirasawa, B., Takeuchi, Y., *et al.* (2016). Autologous preconditioned mesenchymal stem cell sheets improve left ventricular function in a rabbit old myocardial infarction model. *Am J Transl Res., 8*(5), 2222-2233.

65. Lee, J. H., Um, S., Song, I. S., Kim, H. Y. & Seo, B. M. (2014). Neurogenic differentiation of human dental stem cells *in vitro*. *J Korean Assoc Oral Maxillofac Surg., 40*(4), 173-180.

66. Stanko, P., Altanerova, U., Jakubechova, J., Repiska, V. & Altaner, C. (2018). Dental Mesenchymal Stem/Stromal Cells and Their Exosomes. *Stem Cells International, 2018*(8973613).

67. Akintoye, S. O., Lam, T., Shi, S., Brahim, J., Collins, M. T. & Robey, P. G. (2006). Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. *Bone, 38*(6), 758-768.

68. Hatcher, H. C., Atala, A. & Allickson, J. G. (2015). Chapter 2 - Landscape of Cell Banking. In: Atala A, editor. *Translational regenerative medicine.* London: Academic Press, 13-19.