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PERSPECTIVES OF STEM CELL USE IN RECONSTRUCTIVE MAXILLOFACIAL SURGERY

© *M.G. Semenov[1,2], Yu.V. Stepanova[1], D.O. Troshchieva[2]*

[1] The Turner Scientific and Research Institute for Children's Orthopedics, Saint Petersburg, Russia;
[2] North-Western State Medical University n. a. I.I. Mechnikov, Saint Petersburg, Russia;

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The discovery of stem cells is one of the greatest achievements of molecular and cell biology, and associated research has confirmed the possibility of self-renewal and differentiation into specialized tissue stem cells. The use of cellular technologies is an important trend in modern medicine. The aim of this article is to briefly review current findings on the use of stem cells in cardiology, endocrinology, neurology, traumatology, and maxillofacial surgery. All data were retrieved from experimental and clinical studies using various cell technologies. The material is part of ongoing maxillofacial surgery research to investigate the possible use of stem cells in reconstructive maxillofacial surgery for jaw bone pathologies in children. Present tissue engineering methods provide some opportunities for solving difficult clinical problems in oral and maxillofacial surgery. Despite some international achievements of effective application of IC in various diseases, clinical use in reconstructive surgery requires further investigation..

Keywords: tissue engineering, stem cells, reconstructive surgery.

ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ СТВОЛОВЫХ КЛЕТОК В РЕКОНСТРУКТИВНО-ВОССТАНОВИТЕЛЬНОЙ ХИРУРГИИ ЧЕЛЮСТНО-ЛИЦЕВОЙ ОБЛАСТИ

© *М.Г. Семенов[1, 2], Ю.В. Степанова[1], Д.О. Трощиева[2]*

[1] ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России, Санкт-Петербург;
[2] ФГБОУ ВО «СЗГМУ им. И.И. Мечникова» Минздрава России, Санкт-Петербург

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Резюме. Открытие стволовых клеток является одним из крупнейших достижений молекулярной и клеточной биологии, так как исследованиями была доказана возможность самообновления и дифференцировки в специализированные ткани стволовых клеток. Использование клеточных технологий является одним из актуальных направлений в современной медицине. В статье проведен краткий обзор современных данных об использовании стволовых клеток в кардиологии, эндокринологии, неврологии, травматологии и челюстно-лицевой хирургии. Представлены данные экспериментальных исследований и клинических испытаний с применением различных клеточных технологий. Приведенный материал является частью исследования челюстно-лицевых хирургов по изучению возможности применении стволовых клеток в реконструктивной челюстно-лицевой хирургии патологии челюстных костей у детей. Методы тканевой инженерии предоставляют определенные возможности для решения трудных клинических задач, в том числе в челюстно-лицевой хирургии. Несмотря на определенный мировой опыт эффективного применения стволовых клеток при различных заболеваниях, клиническое применение в реконструктивной хирургии требует дальнейшего изучения.

Ключевые слова: тканевая инженерия, стволовые клетки, реконструктивно-восстановительная хирургия.

Perhaps, the greatest contribution to discovery of stem cells (SC) was made by Alexander Alexandrovich Maksimov (1874-1928), the Russian scientist, histologist, and the propounder of the unitary theory of hematopoiesis. Maksimov noted that over the lifetime of a human body, poorly differentiated cells, which are approaching transformation into embryonic mesenchymal cell lineage, are retained around the small vessels. Later, he called these cells as the "stem cells", a term that refers to their location in the "trunk", which is the base of the hematopoietic tree [1, 2].

A. Y. Friedenstein and I. L. Chertkov discovered two types of multipotent stem cells in the bone marrow (BM MSC): hematopoietic stem cells (HSC), the precursors of all blood cell types, and the stromal (mesenchymal) stem cells (SSC) that rarely divide. Their findings are summarized in the monograph "Cell fundamentals of hematopoiesis (hematopoietic progenitor cells") [3-5].

An article by James Thomson (USA, 1998) published in the journal Science surmised the possibility of isolation of embryonic stem cells (ESCs) from the human blastocysts. Simultaneously, similar studies were published by John Gerhart (USA). According to the journal Science, isolation of ESC and their in vitro cultivation (propagation in a culture medium in special vials) was the third most important discovery in biology after the decoding of the DNA double helix and the completion of the mapping of the human genome [3].

The concept of stem cells. Stem cell refers to a progenitor cell with the ability to self-renew and differentiate into specialized tissues.

In the literature, stem cells are classified [4, 6-10] on the basis of their ability to differentiate or on the basis of their origin.

According to their ability for differentiation, there are following types of stem cells:

– totipotent cells, which differentiate into a complete organism. These refer to the embryonic cells and the cells of the extraembryonic structures before implantation (11th day after fertilization);
– pluripotent cells, which differentiate into various organ or tissue elements. These are embryonic cells from the post-implantation period to the 8th week, inclusive;
– multipotent cells, which are the precursors of cells of several types;
– unipotent cells, which retain the ability to differentiate into only one type of cells.

The following types of stem cells are distinguishable on the basis of their origin:

– embryonic stem cells – these are stem cells isolated during the early stages of embryogenesis (at the stage of blastocyst or the germ of the 5-week-old embryos) or teratocarcinoma (tumor line) *in vitro*;
– fetal stem cells are found in the umbilical cord blood and the placenta and are differentiate into different cell types;
– stem cells found in the adult organism: hematopoietic stem cells in hematopoietic organs and blood, which differentiate into different germs of hematopoiesis. Mesenchymal (stromal) stem cells are located in the bone marrow; they are capable of differentiation into osteoblasts, chondrocytes, tenocytes, adipocytes, myoblasts, and fibroblasts. Stem cells are also present in other tissues (regional SC), such as the skin, blood vessels and nervous tissue, and these retain the ability to differentiate into corresponding cell types.

ESCs are considered the most primitive stem cells which are formed by the 5th day after fertilization, as the inner cell mass. These have a high potential to differentiate into and reproduce other types of cells. The unresolved legal and ethical issues associated with their use and the lack of ability to harvest ESCs suitable for clinical use has constrained the use of ESCs. Furthermore, ESCs express most of its genome "in random manner".

ESCs enabled for the first time the verification of the source of chondrocytes. In the course of embryogenesis *in situ* the predominant cell mass of cartilage tissue is formed from the mesenchymal stem cells (MSCs). Under influence of a certain set of signals, cultured totipotent cells of teratocarcinoma of the ESC line of mice were shown to differentiate into chondrocytes and adipocytes [11]. Under the influence of certain other signals, totipotent human MSCs were shown to readily differentiate into adipocytes [12].

The scientists actively conduct the studies in the field of obtaining pluripotent somatic cells of animals and humans. Two basic ways of achieving this are described. The first involves the use of cloning methods such as cell fusion, transfer of somatic cell nuclei in the oocyte of the second meiotic division

(somatic cell nuclear transfer, SCNT) [13]; the second method involves reprogramming to obtain induced pluripotent stem cells (induced pluripotent stem cells, iPS cells) which are similar to ESCs [13, 14]. In 2006, Japanese scientists managed to obtain the first SCs which do not differ in their properties from ESCs of mammals.

Studies in the field of tissue engineering and tissue regeneration are performed using the embryonic stem cells, induced pluripotent and somatic stem cells. However, autologous MSCs isolated from bone marrow, adipose tissue, skin, umbilical cord and placenta have found immense clinical application, the seminal impact of which is reflected in the tens of millions of therapeutic transplantations performed for various diseases. Embryonic stem and induced pluripotent cells are not used in clinical practice due to a number of limitations which include their genomic instability and tumorigenicity.

The International Society for Cellular Therapy (ISCT) has established minimum criteria for characterization of MSCs [15]. MSCs is a group of heterogeneous cells, adhesive to the plastic substrate, which are isolated from bone marrow, adipose tissue, placenta, umbilical cord blood or any other tissue. The name MSC merely connotes the mesenchymal origin of these cells and does not necessarily imply a restricted potential for differentiation. Four stromal cell populations are heterogeneous, and only a small number of these have properties of stem cells or those similar to them" [16, 17].

MSCs can be isolated from various tissues, such as muscle and embryonic connective tissues [18]. However, the ability to isolate MSCs from adipose tissue in virtually any amounts from individuals with different body types is considered the most promising, for example during lipoaspiration [19]. About 2-6 × 10^8 cells can be obtained from 1 cm^3 of adipose tissue. More than 300 cm^3, that is about 150 × 10^9 karyocytes can be isolated from one donor. Such a volume of MSCs is adequate for therapeutic use without the need for prior cultivation. Further, the concentration of the desired phenotype MSCs can be increased during cultivation by a factor of hundreds. To isolate a set of osteogenic precursor cells, beta-glycerophosphate (donor of inorganic phosphate), ascorbic acid and dexamethasone are added to the standard culture. Similarly, insulin, beta-transforming growth factor and ascorbic acid are used for chondrocyte growth [19].

Due to their multipotency, SCs are able to differentiate into different lineages, osteogenic, chondrogenic and adipogenic, that can be used for the development of new cellular biomedical technologies. The ability to produce a wide variety of cell types makes them the most important restorative reserve in the body, which is used for defect replacement.

Clinical application. Use of SC is one of the most promising developments in modern medicine. A considerable amount of scientific studies have demonstrated the high efficacy of the use of SCs in a number of diseases, including those that affect the maxillo-facial area. At the same time, the direct application of SCs raises many questions related to statutory compliance, the risk of malignancy in the process of their cultivation and during the post-transplant period, as well as those that relate to the regulation of cell differentiation [20].

The Ministry of Health of the Russian Federation considered the next version of the draft of the federal law "On circulation of biomedical cell technologies" (Federal Law "On biomedical cell products" dated 06/23/2016 number 180 FZk) [21, 22], which regulates the relations that arise in connection with the development, preclinical and clinical studies, examination, state registration, production, storage, disposal, use, the application monitoring, import in the Russian Federation, the export of biomedical cell products from Russia.

Each MSC pattern used for therapeutic purpose is evaluated from the perspective of the presence of favorable biological properties and the safety of their use in a particular clinical situation. The purity of the resulting culture is assessed by MSC immunophenotyping. Then the biological properties and safety of the MSCs obtained *in vitro* is evaluated against a number of criteria. The proliferative potential of the cells obtained is assessed (culture techniques, cytochemical methods for determining the aging of cells, molecular-genetic methods), the ability of multipotent mesenchymal stromal cells (MMSC) to differentiate in vitro and the expression of genes involved in differentiation is evaluated. The interaction of MMSC with other cells is evaluated as is their genetic stability and tumorigenic potential (cardiological examination with the detection of genomic and chromosomal aberrations, molecular and genetic study is conducted for the detection of mutations in genes and oncogenes and

the tumor suppressors). Finally, the microbiological safety is assessed (bacteriological and virological studies) [23, 24].

The ability of MSCs to differentiate is their essential feature. The differentiation potential is determined in the course of cell culture under special conditions, with detection of specific changes in the direction of differentiation [25].

For the treatment of neurodegenerative diseases and rehabilitation of patients with acute and chronic disorders of cerebral circulation the ability of MSCs to undergo neuron-like differentiation is of much interest [26].

The prospects for use of MSCs with ability for cardiomyogenetic differentiation for regenerative therapy for cardiovascular diseases are being evaluated [27]. In recent experimental and clinical studies, the effect of MSCs on the course of cardiovascular diseases was shown to be mediated via their paracrine effect, i.e., by production of cytokines that promote sustainability of cardiomyocytes under hypoxic and apoptotic conditions [28]. Use of the bone marrow SCs for reconstitution of human heart valve tissue is envisaged. This technique holds immense promise for the future to grow from the SCs of a full heart for transplantation to patients (UK, 2007). Full-structure capillary blood vessels are grown from human embryonic SCs (Japan, 2004). AngioStem technology for cord blood SC transplantation for restoration of blood vessels, and clinical studies of the treatment of peripheral arterial diseases with autologous bone marrow SCs were conducted (Indiana Center, 2007) [29]. The most effective methods of transplantation of autologous myoblasts and bone marrow SCs are in the heart tissue. Medical centers in Europe, Asia, the US and Russia have experience of transplantation.

Neonatal MSCs were found to have an ability to differentiate in hepatocyte-like cells, which can also be obtained from adult sources. The possibility of the use of MSCs for histotypic restoration of liver tissue is studied [30]. According to the studies, MSCs increase the trophism of hepatocytes and reduce the level of apoptosis by their paracrine effect [31]. The ability to differentiate into the cells of the pancreatic islets enables the use of MSCs in the treatment of diabetes mellitus. Due to their immunomodulatory properties, therapeutic MSCs transplantation in autoimmune type I diabetes is possible [32, 33].

Use in orthopedics, traumatology, maxillo-facial surgery and dentistry. The osteogenic differentiation of MSCs involves the ability of these cells to develop into osteoblasts, which is promising for the use of MMSCs in providing reparative regeneration of the bone tissue, including for the treatment of imperfect osteogenesis. To induce the osteogenic differentiation of MMSC, dexamethasone, ascorbic acid and β-glycerophosphate is added to the culture medium. MMSC differentiation into osteoblasts is confirmed by the expression of alkaline phosphatase and the presence of extracellular calcium salt precipitates [15, 34].

A study by P. Janickietal et al. (2011) revealed that MSCs of different donors have different osteogenic potential. The osteogenic potential of MSCs obtained from various tissue sources is also different. The evaluation of osteogenic potency was performed by determination of alkaline phosphatase in the cell lysate of the MSC culture on Day 7, 14 and 21 of osteogenic induction. The chondrogenic differentiation of MSC opens the prospects of clinical application of the cells to restore cartilaginous tissue, especially of articular cartilages. The chondrogenic differentiation of MSCs is inducible *in vitro*, by the addition of basic fibroblast growth factor (bFGF), TGFβ3 and bone morphogenetic protein-2 (BMP-2) to the culture medium. Chondrogenesis can be confirmed *in vitro* on staining with gossypimine or alcian blue, to determine glucosaminoglycan content, which is the major component of the amorphous substance of the cartilage extracellular matrix [35, 36].

The individual use of MSCs in traumatology and orthopedics to supplement their deficiency or to stimulate bone formation has not found wide application till date, as it appeared to be inefficient. However, the biomimetic principle can be used to further develop their use [37]. According to it, to restore the bone defect the hybrid implant is used, which consists of a carrier (hydroxylapatite, tricalcium phosphate, collagen, polymers, and others), MSCs and a growth factor, predominantly BMP and/or TGF [38-40]. The experiments on ectopic bone formation show that this mechanism can operate in direct and indirect mode. In the first case, it contains exogenous MSCs, and in the second case, the SCs migrate from the surrounding tissues and accumulate on the carrier [41].

According to some authors, SCs can be used for the treatment of severe forms of arthrosis, until full recovery. Stem cells enhance blood supply to the diseased joints and improve the body metabolism. Joint pain in arthrosis is significantly reduced when SCs are administered intravenously. On local administration of SCs in the joint the pain syndrome can be completely arrested. The efficacy of SCs in treating arthrosis and concomitant diseases approaches an average of 85%, with the results clinically evident immediately. SCs have found application in the treatment of spinal osteochondrosis, via activation of regenerative processes in the affected vertebrae [42, 43].

MSCs are used more often in adult patients with musculoskeletal disorders. In case of violation of the integrity of the cartilage cover after injury or as a result osteochondropathy, an early degenerative-dystrophic damage of the joint occurs. Local administration of cells by injection into the joint cavity with 1-2 mL of patient's blood plasma or the intra-articular fluid taken from a patient's healthy large joint. The minimally invasive method supplements the articular cartilage defects, creates conditions for optimization of reparative processes in the most severe and frequent damages to the joints. Also, this method is used in combination with surgical treatment, which involves MSCs transplant. During the treatment the cartilage grows in size, receives adequate nutrition due to diffusion of substances from the synovial fluid, and allows complete replacement of the hyaline cartilage defect (the materials from the stem cell bank of Pokrovskaya hospital, C). Similar method was developed and is used in patients with pathology of large joints at the N.N. Priorov Central research institute of traumatology and orthopaedics in Moscow. Osteotomy of shin bone and calf-bone was performed with their subsequent distraction. During osteotomy, the patient's bone marrow was obtained. In the laboratory, MSCs were isolated and cultured from the bone marrow. Three weeks after the osteotomy, 5 mL of physiological solution with 5 million MSCs was administered to the target site for regeneration during distraction. The treatment course comprised of five injections at intervals of 5-7 days.

This approach involves the use of a cell culture enriched with MSCs, or the MSC culture cultured *in vitro*. The cell expansion *in vitro* is necessary to obtain an effective dose of MSCs. The clinical efficiency of use of cells is demonstrable in spondylodesis with bone auto-grafting and surface application of a suspension of autologous MSCs with β-tricalcium phosphate. A method referred to as "injectable bone" (BM MSCs in the gel of plasma enriched with platelets) is being developed for simultaneous augmentation of the alveolar process and placing the dental implant [44].

In maxillo-facial surgery, BM MSCs are used to repair the bone tissue more often than the other cell types [34]. The regenerative potential of BM MSCs was demonstrated both in experimental studies [45, 46] and in clinical studies [47]. An important feature of MSCs is their immunosuppressive effect on T- and B-cells and natural killer cells which may be useful in the treatment of pathologies of the mesenchymal tissue and also to suppress the possible inflammatory response to components of the tissue engineering product. Y. Yamada et al. (2008) [48] used autologous BM MSCs in their clinical and experimental studies and showed high efficiency of bone defect recovery. In an experiment on dogs [49], this group of researchers created 10-mm deep bone defects on the surface of the alveolar part of the mandible, where the bone bone-replacing materials were implanted. The following series of experiments were performed: use of plasma enriched with platelets (PRP); BM MSCs together with PRP; MSC of a temporary tooth together with PRP; MSC of a dental papilla together with PRP; a control series without bone bone-replacing material. The degree of bone regeneration and the implant resorption was examined histologically at weeks 2, 4 and 8. The control defect and the defect with a PRP-implant showed a low rate of bone formation, while the defects filled with BM MSCs together with PRP, MSC of a temporary tooth/PRP, MSC of dental papilla/PRP showed a good degree of bone regeneration [50].

Donor zones. MSCs can be obtained from adipose tissue, as this tissue is more easily-accessible biological material compared with the bone marrow, which is the main source of MSCs. The material obtained from adipose tissue is better suited for use in traumatology and orthopedics, as it can be differentiated more effectively into bone cells. In addition, adipose tissue MSCs can stimulate the vascular growth through secretion of the vascular endothelial growth

factor (VEGF), which confers greater efficacy (the material from the stem cell bank of Pokrovskaya hospital).

The germ and the pulp of the third molar teeth and temporary human teeth can be used as sources of SCs. This biological material is completely accessible, and the cell populations in are similar to adipose tissue MSCs and are capable of proliferation both *in vitro* and *in vivo* and are multipotent. According to the study results, cells from the human third molar germs have properties similar to those of MSCs, express high levels of mRNA genes of transcription factors, which is characteristic of pluripotent SCs, and are able to differentiate into adipogenic, chondrogenic, osteogenic and neuronal lines.

One of the interesting fields in tissue engineering, including maxillo-facial surgery, is the use of scaffolds. Scaffolds are three-dimensional porous or fibrous matrices that perform the function of a mechanical frame for cells. Such materials include natural polymers (collagen, cellulose, fibronectin, chitosan, alginate and agarose, fibroin), synthetic polymers (polylactide, polyglycolide, polycaprolactone, polyvinyl alcohol) and bioceramics (hydroxyapatite, tricalcium phosphate and bioactive glass). Particular attention has been paid recently to innovative technologies of rapid prototyping, that is processes of formation of three-dimensional object based on a digital model, the most usable of biopolymers are laser stereolithography, selective laser sintering, fused deposition modeling and 3D-printing. In the process of producing bioengineered structures based on scaffolds (SC planting on the matrices prior to their transplantation to the site of defect) bioactive substances that induce osteogenic differentiation and attract new cells of the carrier, as well as stimulate angiogenesis are used. These substances are mainly represented by various growth factors [51-53].

Review of the scientific literature has shown that during the last decade, the attention of clinicians is increasingly drawn to such "ideal" grafting materials as autologous MSCs. Undoubtedly, this material cannot but catch the interest of surgeons dealing with reparative and restorative surgery for pediatric patients. The multipotency of MSCs and their ability to differentiate into chondrogenic and especially osteogenic directions can provide over the long term a virtually unlimited amount of grafting material, which will solve one of the most difficult tasks in reconstructive surgery of the skeleton in children, which is the problem of choice of the autologous bone-replacing material.

For more complete judgment on the regenerative possibilities for tissue engineering in restoration of bone defects, more preclinical and clinical studies are necessary consistently under the control of informative clinical, laboratory and morphological methods of studies allowing to evaluate in dynamics the manifestation and direction of regenerative processes.

Despite the certain international experience of effective application of SCs in various diseases, mainly for therapeutic and cosmetic purposes, their clinical use in surgery requires further research.

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Information about the authors

Mikhail G. Semyonov — MD, PhD, professor, head of the department of maxillofacial surgery and surgical dentistry n.a. A.A. Limberg of the North-Western State Medical University n.a. I.I.Mechnikov. Leading research associate of the of the department of maxillofacial surgery. The Turner Scientific and Research Institute for Children's Orthopedics. E-mail: sem_mikhail@mail.ru

Yulia V. Stepanova — MD, PhD, chief of the department of maxillofacial surgery. The Turner Scientific and Research Institute for Children's Orthopedics. E-mail: turner8ord@gmail.com.

Darya O. Troshchieva — MD, PhD student of the department of maxillofacial surgery and surgical dentistry named after A.A. Limberg of the North-Western State Medical University n.a. I.I. Mechnikov. E-mail: troschieva_dariya@mail.ru.

Михаил Георгиевич Семенов — д-р мед. наук, профессор, заведующий кафедрой челюстно-лицевой хирургии и хирургической стоматологии им. А.А. Лимберга ФГБОУ ВПО «СЗГМУ им. И.И. Мечникова» Минздрава России. Ведущий научный сотрудник ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России. E-mail: sem_mikhail@mail.ru.

Юлия Владимировна Степанова — канд. мед. наук, заведующая отделением детской челюстно-лицевой хирургии ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России. E-mail: turner8ord@gmail.com.

Дарья Олеговна Трощиева — аспирант кафедры челюстно-лицевой хирургии и хирургической стоматологии им. А.А. Лимберга ФГБОУ ВО «СЗГМУ им. И.И. Мечникова» Минздрава России. E-mail: troschieva_dariya@mail.ru.