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## **The morphology and application of stem cells in digestive system surgery**

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### **Abstract**

**Background:** Stem cells constitute a group of cells which possess the ability for self-renewal as well as the capacity of differentiation into a vast number of different cells within the human organism. Moreover, stem cells are able to undergo a potentially unlimited number of divisions and this characteristic is clinically essential. Specific fields of its application include treatment of diseases mainly in the field of hematology, orthopedics, surgery, dentistry, and neurology.

**Materials and methods:** In the following work, the current knowledge concerning mechanisms of stem cell treatment in different parts of the digestive system with its diseases as well as adjacent therapy for surgery has been revised.

**Results:** Stem cells therapy may be used in the treatment of various diseases of different parts of the digestive system. This also applies to the end part of the digestive tract (proctological diseases) because stem cells can be used to treat fistulas. Liposuction allows more recovery of mesenchymal stem cells, compared to previous bone marrow harvesting methods. Despite the application of stem cells in the treatment of different diseases used

for many years so far, the therapeutic use for the regeneration of the gastrointestinal tract is still rare and unfamiliar.

**Conclusions:** Regenerative medicine seems to be a promising tool in medical research, especially when insulated cells and designed biomaterials are taken into consideration. Major points of discussion include type of stem cells, stem or differentiation for the treatment of many diseases.

**Key words:** stem cell, digestive system surgery, fistula

## **INTRODUCTION**

Stem cells constitute a group of cells able to self-renew (i.e., have the ability to undergo a potentially unlimited number of divisions). Stem cells can differentiate into other, various types of cells. These unique attributes allow stem cells to be widely used in clinical medicine in at least three fields, namely I) therapy to replace lost or destroyed cell lines, or to modify the behaviour of other cells; II) targets of drug therapy, and III) growth of differentiated tissue for studying disease models *in vitro* for drug development.

Specific areas of stem cell application allow treatment of different diseases in the field of hematology, orthopedics, surgery, dentistry, and neurology [1]. Stem cells are used as a group of healing agents against cancer next to chemotherapy or hormone therapy (in leukemia, lymphoma, myelodysplastic syndrome and other hematopoietic cancers, as well as kidney and breast cancer or Ewing sarcoma), but they are also widely spread in aesthetic medicine [2-3]. Moreover, stem cells constitute great potential to be applied in regenerative medicine, as well. In this context, the use of stem cells falls within an interdisciplinary area of medicine, and combines knowledge from such branches like tissue engineering and molecular biology, while supporting healing, regeneration and repair of damaged tissues.

In this review, we want to depict the current knowledge and importance of stem cell therapy in digestive system surgery.

## **HEADING**

Cell therapy takes advantage of human cells for the regeneration of damaged tissues or even the whole organs of the patient. The major problem of transplant rejection by the recipient organism can be significantly reduced or even eliminated by creating and transplanting organs which are grown in the laboratory from patient's own stem cells. Stem cells or progenitor cells are often utilized because of their internal regenerative potential for damaged tissues. The presence of stem cells in transplanted tissues increases angiogenesis (vascular formation), reduces inflammation, and prevents cell death. Furthermore, stem cells secrete numerous growth factors into their surrounding environment, including the vascular endothelial growth factor (VEGF), and the tumor growth factor (TGF) [4-5].

There are several types of stem cells, as reported in Table 1.

1. Stem cells are divided into embryonic stem cells and somatic (adult) stem cells. Among the embryonic stem cells there are totipotent stem cells (derived from a multi-cell embryo) which can differentiate into cell of any type, while the pluripotent stem cells can differentiate into any type of adult cell except for placental cells (when derived from the embryonic stem cell). Adult stem cells are multipotential, which may give rise to several different types of cells, usually with similar properties (including hematopoietic cells) or unipotent cells, which may give rise to only one cell type.

Most of stem cells occur in fetal tissues and have the greatest regenerative capacity. Because of ethical reasons, stem cells used for treatment are received from adults. Mesenchymal stem cells (MCSs), which are primarily used, are isolated from almost all tissues (e.g., umbilical cord, periosteum, tendon, skin or muscle). MCSs are easily gained from adipose tissue by liposuction or by surgical excision of the tissue. This method is more effective than obtaining MCS from bone marrow. Intensive research done by academia and industry has focused on mesenchymal stromal cells (MSCs) because of their unique features. Full use of MSCs self-renewing ability is enabled because they MSCs can be easily isolated and expanded through *in vitro* culture [6].

Human mesenchymal stem cells derived from Wharton jelly (hWJ-MSC) are multipotent cells which can differentiate into distinct branches of cells: osteogenic, chondrogenic, adipogenic cells, also can trans-differentiate into neural and glial cells. hWJ-HMSC line presents plasticity and has become an interesting and promising tool for the

treatment of cellular and neurogenic regenerative medicine [7]. In addition, MSCs exert immunomodulatory activity and can differentiate ~~distinguish~~ into different lines, making them highly attractive for clinical applications in cell therapy according to the concept of developmental engineering [8]. Lenas P et. al [9] studied the role of bone morphogenetic protein (BMP) signaling in the differentiation of adult mesenchymal progenitor cells, namely hMSC, towards articular cartilage. The combination of microfluidic system for screening soluble factors on 3D microaggregates and synthetic compounds that selectively and specifically silenced the BMP pathway by targeting ALK2 and ALK3 receptors, successfully developed a strategy effective in targeting the hMSC towards stable articular cartilage, both in vitro and in vivo. Thus, restricting BMP signaling can program adult hMSC to stable chondrogenesis [9].

Stem cell therapy also has an important role in heart regeneration, i.e., mesenchymal stem cells (also isolated from adipose tissue), cardiac cell progenitor cells (CPC) and iPSC cells [10].

Usage of stem cells in medicine begins in 1950s when James Alexander Thomson and his associates of the University of Wisconsin-Madison transplanted bone marrow in order to treat leukemia. Stem cell surgery started in the late 1990s, by Dr. Thompson of the University of Wisconsin and researchers at Johns Hopkins University who reestablished the acquisition from human body tissues. High hopes are placed in MCS, but currently the American Society of Plastic Surgeons, WHO or other organizations do not publish official statistics on trends associated with the use of MCS in reconstructive surgery [11].

The mechanisms of action to use stem cells safely to regenerate the human body, must be further explored. The US Food and Drug Administration (FDA) in 2006 developed procedures to ensure the security of patients using stem cell therapy [12].

So far, stem cell treatment methods have been used in more than 70 diseases, including hematopoietic, cardiovascular [13, 14], tumor [24], metabolic [15], dermatological, immune, nervous, bone-joint, digestive, and some genetic diseases. Stem cells can be available in reconstructive surgery to replenish subcutaneous tissue defects. Applications include cancer treatment, radiotherapy, bone grafting, e.g. cranioplasty (filling of skull lid defects), filling of bone tissue defects, e.g. after resection of mandible tumors, cleft palate, treatment of chronic wounds (pressure sores, diabetic foot or as a

result of ischemia, infection), treatment of deep burns, aesthetic medicine and gastrointestinal surgery.

## **APPLICATION OF STEM CELLS IN ENDODONTICS**

The pulp of a human tooth is composed of a basic substance and cellular components, including odontoblasts, fibroblasts and dental stem cells (DSCs), which are mesenchymal stem cells (MSCs). DSCs include dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), and dental follicle progenitor cells (DFPCs). DPSCs are classified as specialized (postnatal) adult stem cells, differentiated by the effects of external factors, leading to the interruption of the process of self-renewal of DSCs. DPSCs, unlike the MSCs, have a multipotential character, so they can vary to at least three different cell lines, such as osteoblasts, endothelial cells, nerve, and fat cells. DPSCs were first isolated in 2000 from perivascular tissue. It is essential to take into consideration the source of tissue donor cells. In general, the younger and less diverse tissue, the better conditions for obtaining DPSCs. Fully retained third molar teeth seems to be the best tissue sample to use. There are no breeding conditions at the present, that would only increase the number of DPSCs without discriminating. The characteristic properties of DPSCs cells allow them to be widely used in both dental and medical research as well as controlled tissue regeneration. Other options for using DPSCs include treatment of neurodegenerative diseases such as Parkinson's disease, filling bones in orthopedics, and primarily in regenerative endodontics - regeneration of tissues in the newly emerging field of dentistry. Bone defects which are caused of malignancy, congenital deformities, traumas, osteoporosis, iatrogenic (surgical) and periodontal disease can be successfully treated using DPSCs and not only MSCs derived from bone marrow bone as it was in the past.

Regenerative endodontics uses stem cells to treat tooth pulp, perforation of root canal walls and bottom of the chamber, as well as necrosis of permanent teeth pulp with incomplete root formation by revascularization and regeneration. Stem cells are an excellent source of controlled tissue regeneration. Obtaining poorly differentiated cells is difficult, however possibilities of using DPSCs are very promising, therefore scientific research is still conduct.

Stem cells are also used in therapy of different organs of digestive system. Despite using these cells for treatment different diseases for many years, therapeutic use for the regeneration of gastrointestinal tract is still very rare and new [16-20].

## **STOMACH**

Rashed et al. studied the combined effect in rat model of mesenchymal stem cells gained from the bone marrow and nitric oxide inducer on injured gastric mucosa [21]. Administration of either MSCs, NO, or MSCS with NO may exert a therapeutic effect on the mucosal lesion in gastric ulcer. Effects were tested by histopathology after IV injection of MSCs and NO inducer. Mucosal regenerative changes and complete restoration in gastric ulcer occurred in the group receiving both stem cells and NO. Wang et al. studied using the group of 48 clean grade male Wistar rats. The aim was to establish the model of gastric ulcer with acetic acid. Bone marrow mesenchymal stem cells (BMSCs) can accelerate ulcer healing by the secretion of VEGF, and improve the quality of ulcer cure. In the regenerative mucosa, the ulcer area, the mucosal thickness and the number of dilated glands by histology was measured by the authors. It turned out that the expression of vascular endothelial growth factor (VEGF) was detected at ulcerative margin, using immunohistochemical method [22].

## **LIVER**

In animal models BMSC cured cure animal liver fibrosis. Only few studies exist which have been performed on humans. Zhang D. studied 60 patients with liver fibrosis secondary to hepatolenticular degeneration. The efficacy of treatment (penicillamine group vs. combination penicillamine plus BMSCs group) was evaluated based on hepatic fibrosis, liver function, and serological markers. Combination therapy was related to lower cytokine levels, meaning a significant positive effect on liver fibrosis [23].

## **PROCTOLOGY**

Healing fistulas using stem cells is more accessible in proctology. Indeed, liposuction allows more recovery of fat cells, compared to previous bone marrow harvesting methods.

Anorectal abscesses and anal fistula are acute and chronic phase of inflammation of the anal crypt but they are also the result of abscess or rectal fistula. The causes include

inflammatory bowel disease (Crohn's disease, ulcerative colitis), trauma, foreign body, radiotherapy, rectal or neighboring diseases, immunosuppressive diseases and less common causes (endometriosis, radiation, tuberculosis etc.) [25, 26]. Fistulas surgeries should save sphincter muscle without inducing postoperative incontinence. To avoid complications treatment, however, is less radical and cause recurrences. Furthermore, operations that save sphincter muscle apply only to simple fistulas [28]. Patients with high, branched fistulas cannot be operated by traditional surgical techniques. By the stem cell procedure, the excision of walls and light of the fistula includes specially prepared cell suspension and the simultaneous stamping of the internal fistula. The cost of the extra procedure of picking and preparing the autogenous material for the slurry is certainly limited by the method. The method is effective in approximately 50% of the cases, with low invasiveness and repeatability [27]. Complications which can appear are associated with infection of the operated site and may refer to the liposuction stage and the fistula area [28]. The incontinence because of anal sphincter damage may also be a complication of surgical treatment, especially more aggressive. Several studies have been projected to elaborate minimally invasive treatment of rectovaginal and anal fistulas. The properties of adipose-derived stem cells (ASC) significantly enhance a natural healing potency [28-32].

At the beginning of 21st century stem cell therapy of perianal fistulas was introduced. In 2005 phase I clinical study was published, reporting the treatment of Crohn's disease with autologous adipose tissue-derived MSCs (ADSC's) [25]. Later on the same department conducted phase II study, evidenced that fistulas treated with ADSC's showed higher healing rates (71%) in comparison with fibrin glue treatment (16%) [26]. However, only few ADSC's treated patients remained free of recurrence for more than three years in long term observation [27].

In 2012 Herreros et al. performed a multicenter, randomized, single-blind, add-on clinical trial in 200 adult patients from 19 centers. Patients were randomly assigned to receive 20 million stem cells (group A, 64 patients), 20 million adipose-derived stem cells plus fibrin glue (group B, 60 patients), or fibrin glue (group C, 59 patients) after closure of the internal opening. Fistula healing was defined as reepithelization of the external opening and absence of collection >2 cm by MRI. If the fistula had not healed at 12 weeks, a second dose (40 million stem cells in groups A and B) was administered. Patients were evaluated at 24 to 26 weeks (primary end point) and at 1 year (long-term follow-up). Serious adverse events (SAEs) were absent. In treatment of complex fistula-in-ano, a dose



of 20 or 60 million adipose-derived stem cells alone or in combination with fibrin glue was safe treatment. In the comparison of 3 groups No statistically significant differences were found [33].

Damian Garcia-Olmo reported similar findings [34]. They checked the results of stem-cell therapy under a Compassionate-use Program for patients with recurrent anal fistulae. There were found ten patients who had previously undergone multiple surgical interventions that had failed to resolve the fistula. During this research they closed the internal opening and followed by local implant of stem cells in the fistula-tract wall. The main cell type selected for implant was autologous expanded adipose-derived stem cells. Outcome at 8 weeks was classified as response or partial response. Evaluation one year after the intervention confirmed if complete healing of the fistula occurred. No adverse reactions or complications related to stem-cell therapy occurred during the study period. Both studies independently of each other proved that stem cells are safe and useful for treating anal fistulae [34,35].

A recent multi-center phase III study by Panes et al. showed that 50% of the patients receiving ADSC's treatment had 6 months remission, compared to 34% of patients on placebo treatment [36]. Dietz et al. showed complete recovery in 83% of treated patients [37]. A meta-analysis performed by Lightner et al., which analyzed severe phase I,II and III trials, reports healing rates ranged from 27%-83%, with positive early results in more than half of analyzed studies [38].

## **GASTROINTESTINAL TRACT**

Recently, results of first human Phase 1/2 trial using genetically modified MSCs for treatment of adenocarcinomas of the hepatobiliary and gastrointestinal tract was published. During the progression of the tumor, MSCs are recruited into the stroma of the tumor. That process causes its growth and metastasis [39-42]. Mesenchymal stem cells have several abilities, which make them perfect vehicle for tumor directed therapy. They have the ability to migrate into deep layers of the tumor microenvironment and differentiate into the tumor stroma cells. Moreover, they have low immunogenicity and can be easily isolated and multiplied and [43-46]. The therapy was confirmed to be acceptably safe and tolerable, which was consistent with Phase 1 study results [47]. Study presented signs of

activity with stable disease in five of ten patients, however no impact on tumor markers and size was observed [48].

## WOUNDS

Process of wound healing involves multiple growth factors and stem cells and should be studied both in terms of research and clinical relevance. One of the surgical challenge is the problematic wound healing and ulceration in radiation-damaged tissue. These problems may appear in non-healing surgical wounds, especially in patients with cachexia or with cancers after radiotherapy. Some ongoing clinical trials with growth factors have already produced very promising therapeutic results which can be used in earlier mentioned cases [50-54].

Grafting fat tissue in an irradiated area can improve the quality of the skin and has regenerative effects. The case study by Mohan and Singh describes presents healing of a chronic ulcer resistant to other ways of treatment using fat tissue transfer (which contains adipose-derived stem cells). A 67-year-old woman lady with a chronic, non-healing ulcer in her leg had a squamous cell carcinoma excised, followed by radiotherapy. After every od multipleexcisions,ulcer continued to cause symptoms. The patient underwent a wide local excision and split-thickness skin grafting. Afterwards fat was infiltrated around and under the ulcer. In the histological examination there was a post-radiation dermatitis ulcer with no evidence of malignancy. After a period in which dressings were used, a reduction in ulcer size were observed and more fat was infiltrated around the lesion. Two months later, the ulcer had been fully healed without recurrence. This case shows the potential of adipose tissue to improve damaged skin. Its use will be able to change the need for complex surgical procedures [49].

This kind of cases prove that modern therapies, especially cellular and protein therapies, are the future of skin treatments such as burns and chronic wounds. The increased amount of growth factors associated with inflammation, which is often seen in chronic wounds, may also carry a potential risk of cancer transformation. Cellular and humoral mechanisms of neoplasm has many similarities with physiological wound healing (stem cell involvement, inflammation strong proliferation, similar growth factors,). However, proper wound healing is strictly controlled and after it is completed, it begins a

natural self-limitation process which is completely different compared to the neoplasm process [50-54].

## **SUMMARY**

One of the most promising directions in medical research is regenerative medicine, which main tools are insulated cells and specially designed biomaterials. Discussions in recent years deal with the type of stem, stem or differentiation that will be most useful in the treatment of many diseases but there is no clear answer. However, it is obvious that different types of cells will play a different role in relation to the tasks they are supposed to fulfill. Bone marrow have been introduced into clinical practice cells faster than other types of cells. The ability to differentiate mesenchymal bone marrow stem cells into many cell types makes their use an attractive cell source for tissue and organ regeneration. However, the challenge still remains to effectively differentiate MSC cells towards the desired cell lines and to maintain the phenotype of previously differentiated cells. Fully verifiable results of stem cell treatment are still growing [53,54].

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**Table 1.** Various types of stem cells

<b>Embryonic stem cells</b>	<b>Adult (somatic) stem Cells</b>	<b>Induced Pluripotent Stem Cells (Artificial)</b>	<b>Fetal Stem Cells</b>	<b>Amniotic Stem Cells</b>
Totipotent	Hematopoietic Stem Cells	-	Proper Fetal Stem Cells	-
Pluripotent	Mesenchymal Stem Cells	-	Extraembryonic Fetal Stem Cells	-
Multipotent	Neural Stem Cells	-	-	-
Oligopotent	Epithelial Stem Cells	-	-	-
Unipotent	Skin Stem Cells	-	-	-