# Adipose-Derived Stem Cells:

## Innovative Therapeutic Approachs

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# PREFACE

Adipose-derived stem cells (ADSCs) have become a valuable resource because of their abundance and ease of isolation. It is evident that ADSCs may provide a realistic, therapeutic modality for the treatment of any disease. After, bone marrow, umbilical cord blood and the third molar, scientists have look for stem cells in human fat tissue, and they have discovered that there are much more stem cells in human fat tissue than in any other resource. In vitro studies done on these cells show that direct stem cell soybean optimization can be done from these cells depending on many variables. Part of the most important population of adult stem cells, mesenchymal stem cells (MSCs) are full-featured cells that reside usually in blood vessel walls and they participate in all rehabilitative functions. They form both such different tissues as bones and cartilage, and they take charge of increasing blood build up in the wounded area and of speeding the healing process. Recent studies have contended that there are 300-500 times more stem cells in 1 ml fat tissue than in bone marrow. Although there are stem cells in every tissue in

the body and although stem cells have been obtained from such tissues as heart muscle, brain, and bone marrow, the fat tissue has proven to be the most prolific on this issue. We aim is to revise existing literature and adipose-derived stem cells use in applications so as to contribute to scientific research.

## CONTENTS

## INTRODUCTION

Cells that can divide for a long time in the living body, renew themselves, are not specialized and can differentiate according to the needs of the body and turn into other tissue cells are known as "stem cells". These cells are stem cells that renew themselves by dividing, keep their numbers constant, and are intended to be used for replacement and regeneration, which are the main cells that make up all the tissues and organs in our body [1]. Adult stem cells, stem cells derived from cord blood and embryonic stem cells are the three basic stem cells known today. Adult stem cells are found in many tissues and organs in the body, and in case of damage to the cells in the area where they are located, they multiply and ensure the repair of the damaged part. Stem cells are self-regenerating and undifferentiated cells that can transform into many specialized cell types when they receive appropriate signals in body and laboratory settings. Stem cell is defined as " functionally undifferentiated cell with heterogeneous reproductive potential ". According to another definition, the stem cell is a primitive cell that

renews itself by dividing, keeps its numbers constant, forms specialized organs such as blood, liver and muscle, and is capable of differentiation [2]. Stem cell studies started with hematopoietic stem cell discovery in the 1960s. This was followed by the presence of stromal stem cells (mesenchymal cells). In the 1990s, scientists detected nerve stem cells in the mammalian brain. In later years, the presence of stem cells in the epidermis, liver and many other organs has been scientifically proven. Adult stem cells, stem cells derived from cord blood and embryonic stem cells are the three main sources of stem cells known today. These cells have the potential to transform into very different specialized tissue cells when they are stimulated with special biological signals as well as they can differentiate into specialized cells of the tissues they originate from [3]. One of the most important features that distinguishes stem cells from progenitor cells is that stem cells not only produce the cell that will turn into a progenitor cell, but also create their own backup during division. This event occurs as a result of asymmetric cell division and ensures that the stem cell pool remains constant throughout life. The extracellular asymmetry of

stem cells is fulfilled by the microenvironment (niche) outside the cell. The extracellular matrix components that make up the niche, neighboring cells and secretory proteins control the number of stem cells and the state of the cell. The removal of stem cells from the niche results in the rapid loss of self-renewal abilities of these cells. While the proteins secreted from these cells are biochemical determinants, the fluidity of the microenvironment causes the stem cells to be affected positively or negatively as a physical determinant and controls their self-renewal activities. Numerous changes occur in both cellular and extracellular structures in the niche region during development, aging, injury and disease; This affects stem cells positively or negatively.

## General Properties of Stem Cells Difference (Plasticity)

Difference is used to describe a series of changes that cells that make up multicellular organisms undergo in the process of maturation and specialization. Differentiation is a complex set of complex events achieved by the combined effect of cytokines, growth and difference

factors, extracellular matrix proteins and intercellular communications. The cell, which is noticed, is prepared to respond to the signals coming from its environment, while also stopping the division. To do this, it usually reveals enzyme-dependent surface receptors, intracellular receptors and activation pathways, triggering the onset of certain events in the cell. For example, Eiraku et al. (2005) showed that a neuronal stem cell was noticed in glial precursors in the presence of Notch signaling [4]. If the cell expressing the Notch signaling receptor interacts with its ligand, DNER (Delta-notch-like epidermal growth factor-related receptor), the glial cell formation is induced. In contrast, some oncogene products may reverse discrimination; In this way, an adult cell can acquire pluripotent property and turn into a malignant tumor cell. Cutaneous Kaposi's Sarcoma is a tumor tissue caused by human herpesvirus 8 and is one of the diseases that indicate AIDS (Acquired Immune Deficiency Syndrome). The forward recognition process for a cell usually starts from the point at which the proliferation process of that cell ends. Therefore, both processes generally do not occur at the same time. The cell in question reaches a sufficient

number in the proliferation process, then the cell surface and intracellular pathways related to proliferation (ie self-renewal process) are usually closed and mechanisms for recognition are activated. During this process, the cell leaves the division cycle permanently or temporarily and enters the G0 phase. Ensuring that the stem cells are noticed in a certain line or directed differentiation in the laboratory; It is accomplished either by fulfilling certain chemical and physical conditions or by directly modifying the genetic program of the cell. For example; Natural hormones and artificial chemicals such as dexamethasone, indomethacin, isobutyl methylxanthin and insulin are added to the culture medium to differentiate an adult stem cell into the fat cell. Although it is not known whether these substances stimulate the transformation of stem cells into fat cells in vivo, the fat cells obtained in this way usually mature in a few weeks compared to their in vivo counterparts. Similarly, when dexamethasone, ascorbic acid and β-glycerophosphate are added to the culture medium, osteogenic differentiation is provided [5].

## Back-Difference and Stimulated Pluripotent Stem Cells

Another way to differentiate in vitro is to genetically reprogram using various vectors, such as viral or plasmid. Stimulated pluripotent stem cells are obtained in this way. Somatic cells, by using various viral or non-viral vectors, activate genes specific to Oct3 / 4, Sox2, klf4, c-Myc and similar embryonic stem cells, providing back differentiation [6]. Intermediate difference is the difference of the cell that has been noticed in one direction towards another. In the Wolf repair event that takes place in the eyes of the salamander, iris cells are noticed to form the lens by removing the lens in the eye. The concept of intermediate difference is still open to debate, as such examples are often not encountered. However, the concept of metaplasia or beyond difference in pathology can be accepted as an intermediate difference model. In metaplasia, the transformation of some of the gastric epithelial cells or stem cells into intestinal epithelial cells (intestinal metaplasia) can be considered as an intermediate differentiation model [5]. One of the general features of the stem cell is its self-renewal feature. The

stem cell replicates throughout its lifetime, without any specialization, and transforms into organ and tissue-specific precursor cells, if necessary. Stem cells produce the cell that will be noticed on the leading cell during the division, while also making its own backup. This event is the result of asymmetric cell division and ensures that the stem cell pool remains stable for life. Drosophila ovaries show asymmetric cleavage [7].

Asymmetric cell division requires very tight control of both intracellular and extracellular factors together. The destinies of the cells in different microenvironments are also different. The extracellular matrix components that make up the niche, adjacent cells, and secretory proteins control the number of stem cells. For example, the axis of division of stem cells in the Drosophila ovary is determined by the niche; The mitosis shuttle is positioned at right angles to the niche. Thus, while the cell near the niche maintains its stem cell feature, the distant ones are noticeable. Asymmetry in the cell is accomplished by transferring some organelles, protein groups and RNA (ribonucleic acid) to only one of the offspring cells. Some

studies show that DNA is also distributed asymmetrically. At the end of the division, the original DNA goes to one of the juvenile cells and decides, and new DNA synthesis takes place in the other cell, which will turn into a leader cell. Thanks to this mechanism, stem cells are protected from mutations that may occur in the newly synthesized DNA and will cause accumulation and always remain intact as cells with the same genome. Although asymmetric cell division is necessary to keep the stem cell pool stable, symmetric cell division must also occur in order to meet the new cell requirement required in the development process and tissue repair of the embryo. Especially in cases of destruction of tissue functions, this mechanism turns stem cells into pioneer cells and ensures repair in a short time. In addition, stem cells divide symmetrically and form new stem cells [8].

The DNA chains, called telomeres, determine the dividing capacity of the cells at the tip of the chromosomes. The longer the telomeres, the more cells can divide. The activity of the telomerase enzyme, which allows the telomeres to remain long, is very high in stem cells, so they

have a large number of cleavage capacities [5]. The term rootness is used to describe the cellular and molecular properties that distinguish stem cells from other cells. These features, which are accepted as the signature of stem cells, are unique gene expressions or a series of changes after translation, thanks to which stem cells retain their original structure and function regardless. The stem cell type can be determined using markers on the surface of the cells that act as signal pathways or cell-cell adhesion molecules in the cell. Many of these markers were collected under a title as clusters of differentiation (CD, Clusters of differentiation). For example; the most common CD markers CD33 and CD45 for hematopoietic stem cells; for mesenchymal stem cells, it is CD29, CD79, CD105. Apart from difference clusters, transcription factors, enzymes or growth factors are also counted among markers [6]. The classification of stem cells is done in two ways, considering the source and differentiation potential. Stem cells are named as totipotent, pluripotent, multipotent and unipotent according to their differentiation potential. In the second type classification, embryonic, fetal, placenta, cord blood, adult and cancer

stem cells are defined according to the source [5]. Totipotent cells are cells capable of forming an entire organism. Each blastomer totipotent cell in Morula stage can be exemplified (Figure 1) because each blastomer can form individual embryonic and extra embryonic structures (Brook and Gardner 1997). These cells are stem cells that give the embryo, post-embryo all tissues and organs, and non-embryo membranes and organs, and have the ability to unlimited differentiation and go in different directions. All blastomers up to 8 cells (Table 1) are totipotent in the early embryonal period [3].

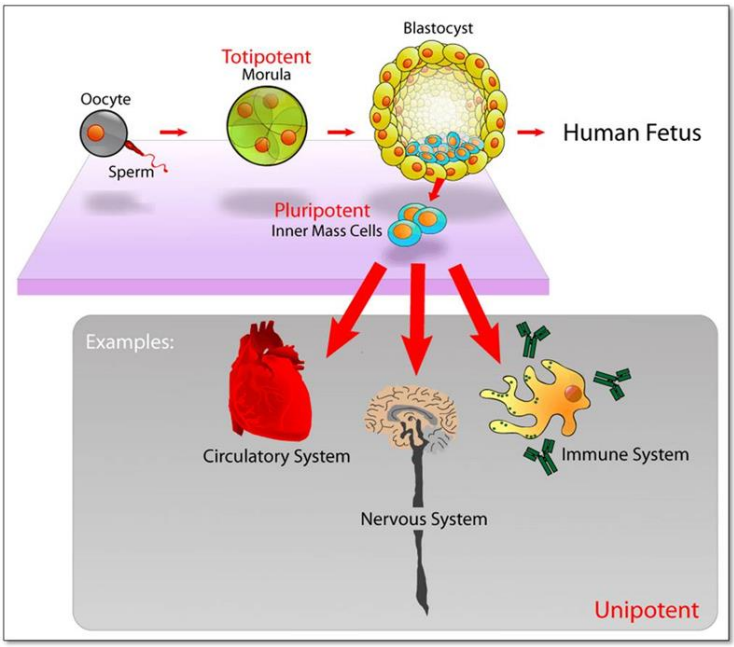


**Figure 1.** According to the potential of differentiation, totipotent, pluripotent and unipotent stem cells (www.koKİMKHcrenedir.com, Accessed date: 12.08.2015).

They are stem cells that cause the formation of many tissues in the organism. After compaction and blastocyst formation, cells in the inner cell mass are pluripotent and these cells have the ability to differentiate many cells of the body (Table.1). In the embryo, the cells in the inner

cell mass of the blastocyst can differentiate into many different types of cells originating from endoderm, ectoderm, and mesoderm (Figure 1). Embryonic stem cells are derived from the blastocyst's inner cell mass and are pluripotent. Embryonic stem cells contain high levels of telomerase activity, no reduction in activation by cell replication. Therefore, they have unlimited proliferation capacity [3].

| İsim | Hücre Tipi (Yerleşim) | Farklanma Etkinliği | Farklanma Yönü |
|---|---|---|---|
| EKH | Morula aşamasındaki hücreler | Totipotent | Embriyo ve embriyo dışı dokular |
| EKH | Blastokist aşamasındaki iç hücre kitlesi hücreleri | Pluripotent | Embriyo gövdesi (Tüm somatik ve germ hücreleri) |
| EKH | Gastrula aşamasındaki epiblast hücreleri | Pluripotent | Endoderm, mezoderm ve ektoderm hücreleri |
| EKH | Ektoderm, mezoderm ve endoderm hücreleri | Pluripotent | Tüm somatik hücreler |
| YKH | Özgün doku hücreleri | Multipotent | Dokuya göre bir veya daha fazla türde hücre |
| YKH | Bir dokudaki yerleşik hücreler | Unipotent | Bir hücre tipi |

**Table 1.** Differentiation aspects of stem cells according to their potential to be different [5].

Multipotent stem cells are cells of the later stage of development and may differ in specialized cell types. Multipotent stem cells are cells that are formed by the

division of these cells and have been programmed to differentiate in one direction. In later stages of development (fetal life), cells have some more specific tasks and turn into adult stem cells. These adult stem cells typically produce cell types of tissue in which they are located. Bone marrow stem cells are the best example. For example, a multipotent blood cell has the ability to transform into other specialized blood cells. Cord blood and adult stem cells are multipotent cells.

Embryonic stem cells are obtained from the inner cell mass in the blastocyst and can differ to any cell type in the organism [9]. Evans and Kaufman succeeded in obtaining embryonic stem cells from the early mouse embryo in 1981. After this study, Thomson et al. First derived human embryonic stem cell lines in the laboratory in 1998. Although these cells first appeared for reproductive purposes in in vitro fertilization methods, they were later donated for use in experimental research. In 2007, the same researchers identified specific conditions that allowed the formation of stem cell-like cells from some specialized adult stem cells by genetically reprogramming

and named them pluripotent stem cells. Embryonic stem cells, which are among the stem cell types, are a stem cell group that is emphasized in tissue engineering and regenerative medicine because of its capacity to transform into all kinds of cells and tissues in living organism [10]. Embryonic stem cells are obtained from embryos that have reached the blastocyst stage in the early development period before implantation. An embryo at this stage consists of two different cell types. The cells called trophectoderm located on the outside form the placental structure after implantation. Cells in the form of a mass in the interior form the fetal structure. Embryonic stem cells are obtained by separating these internal cells using special immunological and mechanical methods and by incubation in environments containing special media and growth factor. Embryonic stem cells are pluripotent cells and, when stimulated with appropriate signals, they have the capacity to turn into approximately 200 cell types in the body. Embryonic stem cells have become the focal point of regenerative medicine thanks to two very important features. These are the capacity to proliferate without being differentiated by the self-renewal process

and the potential to form specialized cell types when they are induced for differentiation [3].

Pluripotency markers are used to identify embryonic stem cells. Of these, Oct4 and Nanog are important molecules. Embryonic stem cells from both human and mouse were found to be Sox2, CD9, CD133 positive. On the other hand, while mouse embryonic stem cells are positive for stage-specific embryonic antigen-1 (SSEA-1), SSEA-3 and 4 are TRA-1-60 and TRA-1-81 negative; In human embryonic stem cells, SSEA-1 is negative, SSEA-3 and 4 are TRA-1-60 and TRA-1-81 positive.

The main research topics in which embryonic stem cells are used in basic sciences are human development, toxicology, and transplantation medicine. However, studies of embryonic stem cells show that these cells are promising for many diseases that are not currently possible to treat in the near future. Thus, diseases that develop due to loss of cells that do not have the capacity to renew and repair themselves can be treated. These include Parkinson's disease, Alzheimer's disease, multiple sclerosis, accidental paralysis and other diseases caused by

the loss of neurons, heart muscle failure, osteoarthritis, bone-cartilage loss, cancer and immune system diseases and diabetes. On the other hand, there are drawbacks in terms of ethical and medical practices regarding the use of embryonic stem cells. Continuous culture of human embryonic stem cells in an undifferentiated step requires animal-based material and nutrient layer. This carries the risk of cross-pathogen contamination. Human embryonic stem cells show high genomic instability and may unpredictably differentiate after long-term development. In addition, differentiated embryonic stem cells can express molecules that can cause immune rejection. It is one of the problems to be overcome before the therapy how the cells that are reproduced in a controlled manner and differentiated to a specific cell type are placed in the appropriate area in the patient and how they are adapted to the appropriate [5]. As a result of the studies carried out in the late 1980s and early 1990s, it has been realized that the umbilical cord and the plesenta are a rich source for hematopoietic stem cells. This issue also supports the development process in the fetus during pregnancy. In the blood of the umbilical cord, which provides the nutrient

and oxygen requirement of the baby by providing the connection between the mother and the baby during pregnancy, in addition to the blood cells such as erythrocytes, leukocytes and thrombocytes, there are stem cells that are higher than adult blood. Cord blood, which was excreted in the old years, can now be used for therapeutic purposes or can be stored under special conditions. The only medically accepted field of use for today is blood and immune system diseases. Since cord blood is in a small volume, approximately 100 ml, the total amount of hematopoietic stem cells it contains is less than that obtained from bone marrow or growth factor-induced peripheral blood. Therefore, umbilical cord blood recipients are typically children. However, when it is realized that the blood taken from several babies can be applied to a single patient recently, it has also been used in adults. The most commonly used case in the world for the moment is the use of stem cell transplant treatment but for the treatment of patients who are not among the family members or who cannot find suitable donors. However, it is necessary to investigate whether the tissue compatibility molecules between the recipient and the donor are

compatible during use. In the family, Human Leukocyte Antigens (HLA) is a fully suitable or at most one antigen incompatible donor, bone marrow / peripheral stem cell ideal donor. If no one with these features is found, non-relative donors come into play. While interpersonal transplants can tolerate an antigen incompatibility, allele level alignment should be achieved in high-resolution typing of both HLA-A, -B, -C and HLA-DRB1 regions. Otherwise, the frequency of Graft versus host disease (GVHD) increases in one allele incompatibility, and the lifespan is shortened in more than one allele incompatibility compared to the most suitable [11]. The placenta amniotic membrane, which provides the physical and functional relationship between the embryo and the mother, consists of chorion and maternal endometrium layers. Stem cells in amnion and chorion originate from the non-embryo mesoderm. Although obtained from all three trimesters, the amniotic membrane mesenchymal stem cells are mostly obtained during childbirth. The amniotic membrane has similar surface markers as mesenchymal stem cells, bone marrow and cord blood derived stem cells. However, unlike other adult

mesenchymal stem cells, they also carry embryo stem cell markers. Because of these features, they have a higher potential for differentiation. 15.-18 of pregnancy There are also stem cells in the amniotic fluid taken by amniocentesis in order to make genetic diagnoses in the weeks. Approximately 1% of the cells from amniocentesis samples contain the c-kit (CD117), which is the stem cell factor receptor, while the other cells are cells that have become different and come from the fetus skin. Cells containing c-kit have been found to be capable of proliferation when separated and cultured by magnetic immune selection analysis. Amniotic fluid stem cells' self-renewal time is approximately 36 hours and does not need a nutritious cell layer. It has been observed that when appropriate signals are provided, amniotic fluid stem cells can differentiate into cells belonging to all three germ leaves. Looking at the characterization of amniotic stem cells, MHC-1 and HLA-ABC are positive for CD29, CD44, CD90 and CD105, while CD34 and CD45 show negative properties [8].

There has been a rapid improvement in the collection and therapeutic application of these cells since the first successful cord blood transplantation in children with Fanconi anemia. The New York placenta blood program center is the largest human cord blood bank in the United States, backed by the National Institutes of Health (NIH). It currently contains about 13,000 donor samples for transplantation purposes for patients who need hematopoietic stem cells. It has started to collect cord blood since 1992 and there are thousands of cord blood units in this center for patients [5]. Adult stem cells can regenerate themselves in the tissues in which they are found, in the event of cell death and tissue damage, and differentiate into specific cells of the tissue or organ in which they are located [12]. The term “somatic stem cell” is also used instead of the adult stem cell. As an organism matures, the number of stem and precursor cells decreases. Thus, tissues in adults contain few stem and precursor cells; these cells are limited to different anatomical locations. Most of the cells in a mature tissue are differentiated cells that have adapted to their environment and have certain phenotypic properties. Consequently, an

organ's regeneration capacity decreases with age and in proportion to the number of stem and precursor cells that can divide effectively. With these limitations, the body has developed two major strategies for replacing and regenerating tissues. In the first way, there is the capacity to multiply in differentiated and functioning cells. Liver, skeletal muscle and vascular endothelial cells are included in this group after migration, where mitogens are released enough to direct limited replacement of cell loss in that area, and thus cell division is stimulated. Examples include bone marrow stem cells, peripheral blood stem cells, mesenchymal stem cells, and adult stem cells located in organs. In addition, neuronal stem cells, dental pulp and stem cells originating from adipose tissue, epidermal stem cells, liver stem cells and stem cells obtained from cadaver are other stem cells located in organs [13]. Hematopoietic stem cells (HKH) are self-renewable bone marrow or multipotent stem cells that can be isolated from blood and differentiate into different types of cells. They can develop into the bloodstream by exiting the bone marrow. They may also be exposed to programmed cell death called apoptosis. Processes such as hematopoietic stem cells to

renew themselves, to remain silent in the G0 phase of the cell cycle, to adhere, to proliferate, to mature, to go into differentiation, to enter the circulation are provided in special microenvironments in the bone marrow. In this area called niche, there are osteoblasts, osteoclasts, stromal cells, extracellular matrix components, molecules, factors, cytokines, which are cells specific to bone marrow, and interactions between them ensure that hematopoietic stem cell functions and hematopoiesis remain constant. Recommended surface markers for hematopoietic stem cells; CD34 +, CD59 +, Thy1 +, CD38 ±, C-kit ±, lin--.. Bone marrow is the classic source of HKH. For more than 40 years, they have performed bone marrow transplantation by pulling cells from the bone marrow, typically by piercing the hip bone with a syringe under anesthesia of the stem cell donor. 1 / 10-100,000 of the cells obtained from the marrow are in the form of stem cells. Other cells are stromal cells, stromal stem cells, progenitor blood cells, mature or maturing erythrocytes and leukocytes. Bone marrow transplant application with the part directly removed from the bone, which was of extreme curiosity in the past, has now been put into

practice with medical use by being thrown from the source of the hematopoietic stem cell for medical treatment. Regarding transplantation in the clinic, peripheral donor stem cell collection is used as a new method. It has been known for many years that there are few stem cells and progenitor cells in the circulating bloodstream. The researchers have found that over the past 10 years, they can inject cytokines such as granulocyte colony-stimulating factor (G-CSF) into the donor to remove a large number of cells from the bone marrow into the peripheral circulation. The procedure is started by injecting G-CSF a few days before the cells are harvested. By placing a tube in the vein by the doctors to the donor where the cell will be collected, CD34 + cell-containing leukocytes are collected by the filter system between them and the erythrocytes are returned to the donor. These collected cells are 5-10% stem cells. Thus, researches commonly prefer peripheral blood in stem cell collection. Actually; peripheral CD34 + cells are actually a mixture of different degrees of mature leukocytes, stem cells and progenitor cells. In the last 3 years, peripherally leukocytes rather than bone marrow are used for

autologous and allogenic bone marrow transplantation [14]. The first clinical uses of HKH include the treatment of blood cancers (leukemia and lymphoma) caused by the proliferation of leukocytes. In these applications, the patient's own cancerous hematopoietic cells are destroyed by radiation or chemotherapy, then replaced by bone marrow product or HKH transplantation collected from the peripheral circulation of the compatible donor, as currently done. The compatible donor is typically a sister or brother with a hereditary similar HLA on the cell surface. Blood cancers; It includes acute lymphoblastic leukemia, acute myeloblastic leukemia, chronic myeloid leukemia, hodgkin's disease, multiple myeloma and non-hodgkin lymphomas. Although there is a significant mortality risk due to both infection and graft versus host disease after transplantation, most patients have increased their lifespan [14]. MKH is an adult stem cell type. The fact that they have a "support cell" feature in general, as they are of stromal origin, constitute the basis of the use potential of MDGs in many fields of medicine Mesenchymal stem cells, which constitute an important part of regenerative medicine today, [15]are produced by

producing the cells obtained under laboratory conditions in petri dishes. They are durable cells that can be obtained from many tissues and are capable of reproduction in number [16]. The soluble factors that they secrete contribute significantly to the functions of the tissue-specific cells in which they are located due to their close relationship with the intercellular or extracellular matrix. They are of great interest because they are important components of the tissue microenvironment and mostly have suppressive properties on the immune system [17].

The necessity that the mesenchymal stem cells must be replicated in vitro cell culture medium due to the very small number of tissues from which they are obtained is the main disadvantage of these stem cells in basic science research and clinical use. This situation leads to differences in phenotypic, immunological and other biological features with the effect of various stimuli and factors that cells are exposed to as a result of passages in the culture medium [18]. Since almost all of the basic studies with mesenchymal stem cells are used in in vitro culture medium, it is known that the defined properties of

these cells are far from reflecting the in vivo properties, even if studied in detail. This poses a disadvantage especially for clinical applications. There is a risk of cell aging due to passage in the culture medium, cytogenetic disorder and malignant transformation, albeit low. At the same time, difficulties in establishing cell processing laboratories in accordance with internationally accepted accreditation conditions for the development of cells suitable for clinical use constitute an obstacle to the widespread use of these cells in the clinic [19]. On the other hand, no serious problems related to cell use have been reported in the clinical applications of MDG, which have been increasing since the mid 2000s, but still few. MDGs are the main cells of the connective tissue. Fat can differentiate into cells such as bone, cartilage, muscle, tendon, ligament. In addition, they constitute the origin of stromal cells, which are supportive cells in all tissues [20]. These cells were first described by Fridenstein in 1 year. Fridenstein showed that bone marrow cultures using fetal calf serum (FCS) have cell colonies that show adhesion ability, morphologically similar to fibroblasts, and have the ability to differentiate into bone and fat cells. In the

studies carried out years later, it was revealed that these cells are pluripotent stem cells that are not hematopoietic, and have the ability to differentiate from cells originating from all three germ leaves. These cells, formerly called CFU-F (Colony forming unit fibroblast) and "Bone marrow stromal fibroblasts", were later identified as mesenchymal stem / stromal cells [21].

Stem cells can be autologous or allogenic and can be administered systemically or locally [22]. There are sometimes contradictions in identifying the typical features of mesenchymal stem cells among researchers. Many laboratories use various methods to isolate MDG and to reproduce and direct these cells to differentiation by following protocols that do not contain significant differences. MDGs with morphologically and biologically similar properties can be isolated from different tissues. However, it is reported that there are changes related to the environmental conditions under which the cells are developed in subjects such as differentiation and immunomodulatory properties of cells, and their effectiveness in vivo. For these reasons, the International

Association for Cellular Treatment (ISCT, UHTD) has proposed the criteria for defining human MDDs for both basic research and pre-clinical studies. These cells were proposed by UHTD to be called "mesenchymal stromal cells" or "multipotent mesenchymal stromal cells / MSC" instead of being called "stem cells". However, in various studies, the ability of cells to transform into different cells of endodermal and ectodermal origin besides connective tissues still causes these cells to be referred to as “MDH” by many researchers. The main features commonly used in defining MDG are; Adhesion to plastic surface (plastic adherence) is the expression of surface antigens in stromal character and the potential for multipotent differentiation [23]. Bone marrow, one of the richest stem cell sources of the organism, is considered to be the main source for MDGs. In the bone marrow, there are hematopoietic, endothelium and mesenchymal stem / projector cells originating from mesoderm. Different studies have shown that bone marrow aspiration has an average number of MSHs ranging from 1 to 10 mononuclear cells, ranging from 2 to 100 [24]. Besides bone marrow, MKH can be isolated from many tissues. Enzymatic methods are used

in cell isolation from solid tissues. It is possible to separate bone and periosteal, muscle tissue, pulp and maxillofacial tissues, liver, lipoaspiration materials, cord blood, cord stroma, placenta, amniotic fluid, synovial fluid and even peripheral blood due to their adhesion properties [25]. Mesenchymal stem cells have many features, regardless of the tissue from which they are obtained, such as adhesion to plastic tissue culture dishes, exhibiting fibroblastoid morphology, versatile differentiation, and some surface markings. These features are largely similar. However, it has been shown that there may be some changes in the differentiation capacity and functional features depending on the type of tissue originated. Depending on the microenvironment they are in and how they are needed in the organism, there are also significant changes in the biological features and functions of MDGs. In relation to this, it has been suggested that the use of stem cells obtained from that region will have advantages for the repair of a specific tissue [26].

The presence of MSC in peripheral blood is controversial. It has been shown that there are nonhematopoietic cells

with osteogenic differentiation ability and peripheral blood. It is shown that MKH is isolated from peripheral blood in cases of bone fracture and multiorgan failure especially in cases of severe damage. Since studies with mesenchymal stem cells are always in vitro, the placement of cells in the tissues, their niche / niche regions have not been studied in detail; nevertheless, especially in recent studies, it has been reported that the cells are located in the perivascular location in the tissues, such as pericides, and coordinate the cellular functions of neighboring cells, such as maturation, differentiation or silence [27].

## Physical Properties and In Vitro Reproduction

MDGs are very few in tissues, including bone marrow. In addition, there are difficulties in obtaining a sufficient number of tissues, depending on the adhesive properties. In order to reach sufficient cell numbers in both clinical practice and basic science researches, they must be reproduced in vitro. It is known that these cells are resistant cells that are suitable for reproduction in vitro and maintain their proliferation and differentiation ability in culture. It is noteworthy that when MSCs reproduced in

culture medium are examined by light or phase contrast microscopy, the cells are spindle-shaped and form fibroblast-like cell assemblies. It is observed that when cells are cultured at low concentrations, they tend to colony formation, but at higher cell density, they multiply in groups of cells arranged next to each other instead of forming a colony [28].

**Stem Cell Microenvironment**

The differentiation capabilities of stem cells are regulated under the influence of indoor genetic pathways and external signals. Stem cells need an environment that supports them and allows these regulatory signals to be transmitted. This microenvironment, called "stem cell niche", contains the cellular and molecular factors necessary for the regulation of cells and control of their functions. In some tissues (like skin), this microenvironment has a regulatory effect on both stem cells and their precursors [29].

Stem cell niches provide support for stem cells, create an environment suitable for their lives, regulate their

proliferations and direct their differentiation. Studies on this subject provide very important information. For example; each niche system uses special molecules such as Notch, which provide physical interaction and cause the asymmetric or symmetric division of the stem cell. Versatile stem cells can be found in a niche. Most stem cells divide acidmetrically to form a son cell that will remain in the niche and a son cell that will leave the niche to differentiate. However, symmetrical division also takes place. Symmetric division of stem cells can ensure the formation of all stem cell lines and the number of stem cells remains unchanged. This is because any decrease is offset by an increase. Stem cells, when tissue damage develops, leave their microenvironment and migrate to the area where the damage develops. Therefore, balancing the number of stem cells in the microenvironment is very important [30].

## Adipose Stem Cells and Their Use in Clinic

Multipotent cells isolated from adipose tissue are called adipose stem cells (ADC). ASCs are obtained from healthy donors by a method based on the removal of

subcutaneous adipose tissue cells from the body by suction with the help of vacuum called liposuction, and they show specific features such as fibroblast-like morphology, easy adhesion and differentiation into mesenchymal cell lines. Under local anesthesia, large amounts of adult fat stem cells are obtained autologously from adipose tissue. It is reported that pluripotent stem cells obtained from adipose tissue have the ability to differentiate as much as stem cells obtained from bone marrow. This situation causes intense interest in many fields, especially tissue engineering [31]. Among the different types of mesenchymal stem cells (MSCs), adipose-derived stem cells appear to be the most promising for cell therapy for several reasons. It has been shown that these cells play a role in the elimination of minor damage in the tissues and organs they are suitable for, and can transform into myocytes and neurons in addition to osteogenic, chondrogenic and adipogenic differentiation, as well as other mesenchymal stem cells, in vitro [32]. Alternatively, mesenchymal stem cells are being differentiated on various bio-materials in tissue engineering and their use in different regenerative treatment areas is studied. There are two different types of

adipose tissue, from which white adipocytes are produced from mesenchymal stem cells (MSCs) extending through the mesoderm and neuroectoderm. MSCs are characterized by their ability to adhere to plastic, grow, and differentiate from mesenchymal stem cell lines into osteocytes, chondrocytes, and adipocytes. MSCs are located in the vascular stroma of adipose tissue, bone marrow, and other tissues [33]. There are many factors that enable or inhibit the differentiation of MSCs on the adipocyte line. Due to the ability of stem cells to renew themselves, to differentiate, to multiply, to form tissues and organs, it is possible to be used as an alternative to organ transplants or drugs with negative side effects in organ transplants and in the treatment of many diseases. Today, stem cells can be obtained from many sources for therapeutic purposes and the most studied sources are bone marrow containing embryonic stem cells, hematopoietic and mesenchymal stem cells. Studies for the treatment of human embryonic stem cells, such as Parkinson's, diabetes, heart failure, medulla spinalis injuries, osteogenesis imperfecta and Purkinje cell degeneration are still at the experimental stage; Because

embryonic stem cells must be differentiated to target tissue cells before transplantation. However, in experimental treatment studies with stem cells, their use in the clinic is suspicious due to the difficulty of monitoring and determining the behavior and cellular mechanisms of these cells [34].

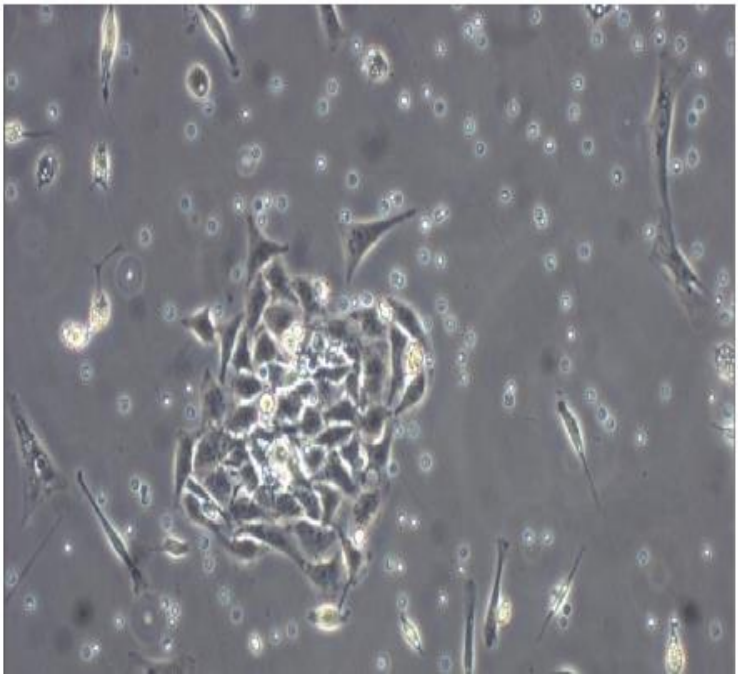

**Figure 1.** Adipose-derived stem cells isolated from adipose tissue. (The picture was taken from the cells we

obtained in our own laboratory. It was not used in any study) [35].

Injury of the heart muscle as a result of obstruction of the coronary artery vessels leads to significant losses in regional heart functions. Transferring cardiomyocytes that will provide cardiomyocyte in the damaged myocardium seems to be promising for patients without treatment options other than heart transplantation. By-pass surgery and reperfusion therapy increase the risk of death, although there are limited treatment options for these diseases. With mesenchymal stem cell therapy, it has been shown that healing can occur due to tissue regeneration as a result of these cells migrating to damaged heart tissue. In addition, the fact that this method does not require a surgical procedure is another reason why it is preferred. Studies are planned to use MDG in degenerative diseases such as developmental anomalies, bone infections, trauma, osteoarthritis and osteoporosis related to skeletal system and tumors. It is considered more appropriate to use mesenchymal stem cells in osteoarthritis caused by trauma

and aging in injuries requiring cartilage and bone repair [36].

MDGs are also seen as an important alternative in the treatment of meniscus. While treatment applications focus on cartilage tissue cells, a lot of experimental research is ongoing for MKH applications for bone and muscle tissue. Because of its immunosuppressive and immunomodulatory effects, MDGs are promising in the treatment of autoimmune disease. Although there are experimental researches on this subject, clinical practice experience is extremely limited today [37]. However, these cells can be used. Today, treatment methods that provide full recovery in a significant part of human nervous system diseases have not been developed. These include multiple sclerosis, neurodegenerative diseases and traumatic nerve cuts [38]. Effective regenerative treatments in spinal cord injuries have been shown in the near future.

Cell-based therapies represent a promising therapeutic approach to enhance the regeneration of damaged tissue and the combination with specific soluble mediators and

biomaterial scaffolds has allowed the introduction of new treatment strategies in regenerative medicine. Adipose tissue is composed mainly of fat cells organized into lobules. It is highly complex tissue consisting more than 90% of the tissue volume and a stromal vascular fraction (SVF) which includes preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/ macrophages, lymphocytes and Adipose derived stem cells (ADSC's) [39]. Adipose tissue like bone marrow, is derived from the mesenchyme and contains a supportive stroma that is easily isolated. Based on this, adipose tissue may represent a source of stem cells that could have far reaching effects on several fields. Putative stem cell population within human lipoaspirate called as processed lipoaspirate (PLA) cells, can be isolated from adipose tissue in significant numbers and exhibits stable growth and proliferation kinetics in culture. The multilineage differentiation capacity of PLA cells led us to speculate that a population of multipotent stem cells, comparable with MSC's can be isolated from human adipose tissue [40].

The field of embryonic stem cell research began in 1981 with the discovery of mouse embryonic stem cells by two independent groups at the University of Cambridge and University of California San Francisco [41]. Thomson et al. from the University of Wisconsin reported on human embryonic stem cells in 1998 [9]. İn 2006, Takahashi and Yamanaka revealed a method for reprogramming differentiated adult stem cells to behave as embryonic-like stem cells, and in turn, these altered cells were termed induced pluripotent stem cells [42]. Stem cells possess the ability to self-renew and differentiate into defined cell types. These cells are the main cells that make up of all the tissue and organs in our body, and they regenerate themselves by division, keep their numbers constant and are presumed to be used for replacement and reconstruction [43]. Stem cells are divided into those derived from the early embryo, called the embryonic stem cells (ESC), and those derived from adult tissue, termed adult stem cells (ASC). There are two main advantages of ESCs versus ASCs, the first being their ability to proliferate for longer periods of time and the second being their ability to differentiate into a broader range of cell

types [44]. However, due to the ethical conflict that surrounds ESCs, their use in preclinical and clinical research has been limited and, as such, is not considered a reliable, therapeutic approach in the near future. Therefore, ASCs have become the main focus of current research, and recent discoveries have shown a greater potential for self-renewal and differentiation in this cell type than initially projected.

Adult stem cells, stem cells obtained from umbilical cord blood, and embryonic stem cells are the three basic stem cells known today. Adult stem cells can be found in many tissues and organs in the body, and if the cells in that region are damaged, they repair the damaged part by multiplying.

One of the most important qualities that differentiates stem cells from pioneer cells is that while stem cells produce the cell that would become the pioneer cell, they also create their reserve during divisions. This happens due to asymmetrical cell division, and enables stem cell pool to always remain constant. Extra-cellular asymmetry of stem cells is performed by the niche that is outside of the cell.

Extracellular matrix components that form the niche, neighboring cells and secretion proteins keep the number of stem cells and the state of the cell under control. Stem Cell Niches are divided into two classes: the stromal niche and the epithelial niche. The stromal niche is defined by localized anatomical locations that contain specialized support cells that maintain stem cell activity. Cell adhesion and extracellular matrix molecules anchor stem cells to the niche and allow for efficient communication between cells through secreted, soluble cytokines, and growth factors [45]. An example that has become increasingly important today is the vascular wall, which has been shown to contain adipose-derived stem cells (ADSCs), hematopoetic stem cells (HSCs), neural stem cells, intestinal stem cells, and testicular stem cells [45]. In contrast, the epithelial niche does not contain this system of support cells, but instead lies at the basement membrane and directly adjacent to mature progeny An example of the epithelial niche would be muscle derived stem cells.

With the advent of studies in stem cell biology and the improvement of techniques in cell culture, stem cell

applications have started to be seen to provide hopeful results in the treatment of such things as cancer, organ deficiencies, metabolic, rheumatic, cardiologic diseases, bone diseases, and nerve damages and nervous-system diseases. Recently, what has become a current issue is using stem cells in the treatment of various diseases that require organ or tissue transfer, and diseases in which existing medical treatments fall short. Although adipose tissue is not practical, recent studies show that this tissue provides a huge amount of adult tissue source. ADSCs can show osteogenic differentiation quite fast. This, in return, brings to mind tissue engineering and the use of adipose stem cells in broad bone defects. In the field of tissue engineering, many studies have been conducted in order to obtain bone marrow stem cell. However, not enough cells can be obtained from the bone marrow. Nevertheless, adipose tissue makes it possible to obtain many stem cells without morbidity. In bone tissue engineering, osteogenic differentiation is achieved by using scaffold materials, and it is believed that they will be used in vitro models in the future.

## ADSCs Clinical Applications

Mesenchymal stem cells have the potential to transform into different cell types such as bone, cartilage and muscle, as well as immune suppressive and trophic effects due to the many growth factors and cytokines they produce. With these features, many studies have been carried out since 1990 regarding its suitability for clinical use. Graft versus host disease is a complex clinical syndrome with organ dysfunction as a result of a severe immunological reaction mediated by healthy T-lymphocytes taken from the donor and given to the patient with stem cells in bone marrow transplants. considered the reason. Mesenchymal stem cells have recently started to be used as an alternative approach in the treatment of GVHD due to their immunomodulatory effects and regulation of immune response. Mesenchymal stem cells suppress T cell proliferation regardless of the basic tissue compatibility complex (MHC). The immunosuppressive and immunomodulatory effects of mesenchymal stem cells are related to the surface molecules they express and contain. Protein fragments from the donor are recognized by the

recipient's T cells. All nucleated cells transmit antigen to T cells through the MHC class I. Mesenchymal stem cells do not cause an immune response because they contain low levels of MHC I class surface molecules encoded by human leukocyte antigen (HLA). Secondly, tissue compatibility is not sought since MSCs generally do not contain MHC II surface molecules found on the surface of lymphocyte and antigen presenting cells. Graft versus host disease is a common complication with high morbidity and mortality that develops due to donor T-lymphocytes attacking different tissues of the recipient after allogeneic hematopoietic stem cell transplantation. Its incidence varies between 20-70% according to the treatment dose, HLA compatibility, age of the recipient and stage of the disease. The standard initial treatment for acute GVHD (aGVHD) is corticosteroids. However, while there is no improvement in 30-50% of the patients, 3rd and 4th stage GVHD may develop, and the risk of toxicity and infection may increase. According to the literature, the survival rate in steroid-resistant GVHD cases is 10% or less.

## Stem Cell Applications in the Treatment of Urinary and Anal Incontinence

The use of stem cells in the treatment of urinary and anal incontinence is mainly based on skeletal muscle regeneration. The urethral and external anal sphincter provide effective continence due to the presence of skeletal muscle. Skeletal muscles have a hereditary regeneration power due to the satellite cells they contain. Stimulated satellite cells form myoblasts. Myoblasts unite to form myotubules, and then these myotubules come together to form the sarcomere structure and form muscle filaments. As a result, this process provides muscle regeneration. Unfortunately, the proportion of satellite cells in skeletal muscle mass is very small. Therefore, major muscle damage or chronic injuries cannot be repaired. Muscle biopsy, in vitro culture, and subsequent transplantation of myoblasts for the treatment of some degenerative diseases were first proposed in 1978. Incontinence models have been developed based on this concept and are more promising because they contain less muscle tissue damage. As stem cell research expands,

theories about its mechanisms of action expand. Originally the theory was the transplantation, fusion, differentiation and functional regeneration of cells. But now potential explanations are: (1) direct stem cell fusion and regeneration; (2) local mass effect; (3) trophic effect by the effect of growth factors secreted by stem cells; (4) includes cell repair with the effect of the immune system. Among the many types of mesenchymal stem cells, adipose-derived mesenchymal stem cells are regarded to be the most promising in cell treatment due to various reasons. It has been shown that these cells play a role in repairing small damages in the appropriate tissue and organs, and that under in vitro conditions they can turn into neurons and myocytes in addition to chondrogenic and adipogenic alteration just like other mesenchymal stem cells. Alternately, mesenchymal stem cells are also researched in terms of their use in various regenerative treatments by altering them in tissue engineering.

Regenerative medicine stands out as the most compelling field for stem cell treatment. Potential dangers of using these cells without optimizing cell preparation methods

and without sufficient clinical experience for burn treatment, for neurological disease, for osteoporosis treatment, for myocardial infarction, for implants in dentistry, and especially for the treatment of diseases which already have alternative treatment methods have been noted. Therefore, reported studies on this fields are fairly limited. Cells therapy promises great hope for difficult to heal wounds. In the new methods using cell molds in tissue engineering, mesenchymal stem cells are placed on these molds and carried to the wound area. In the studies in which mice are applied singular or triple molds, which were obtained from human adipose stem cells, it was observed that there is a faster and more effective healing in the experiment group compared to the control group. In the method which aims to increase angiogenesis in wound area, different cell types and allogenic cell molds are also examined. The hope for staying young has always been fresh for human beings. Many drugs against aging have been manufactured. However, none of them can significantly slow the aging process. Stem cells provide highly important advantages in this respect. Even when we become adults, stem cells

can turn into the organs that are in their program, and can be constantly regenerated. Our skin is one of such organs that are constantly regenerated. With growth factors applied to the skin, it can regenerate itself faster, and this indeed is made possible with the use of stem cell technology by which the transfer of regenerative products onto the skin is accelerated by means of stem cells. Neovascularisation, a key process for wound healing and recovery of ischemia, plays a major role in survival of implanted cells [53]. ADSCs secrete numerous growth factors that take part in angiogenesis and normal wound healing. ADSCs secrete high levels of epidermal growth factors, basic fibroblast growth factor, keratinocyte growth factor, PDGF, insulin like growth factor, VEGF, TGF- Beta, hepatocyte growth factor (HGF), brain derived neutrophilic factor [54]. Growth factors secreted from ADSCs promote wound healing by inducing migration and proliferation of ECs, increasing the vascularity of wound bed, increase vessel density, granulation tissue thickness and collagen deposition [55]. HGF is considered as main angiogenic factor secreted by ADSCs as its suppression impairs the angiogenic and regenerative

effects [56]. İn a study in vivo results showed the effectiveness of using ASCs on reducing the time needed for complete healing to 21.2 days for Sham, 17.4 days for vehicle alone and 14.6 days with the addition of ASCs. İn the event the tolerance and efficacy of cryopreserved ASCs to accelerate the complete closure of the wound by increasing the maturation of the skin and its blood perfusion, shows their therapeutic benefit in the wound healing context [57]. Adipose-derived stem cells (ADSCs) have myocardial regeneration potential, and transplantation of these cells following myocardial infarction (MI) in animal models leads to modest improvements in cardiac function. An other study represented to us, hearts transplanted with ISX1(3,5-disubstituted isoxazoles )-treated ADSCs manifested significant increases in neovascularization, which may account for the improved cardiac function. These findings suggest that a strategy of drug-facilitated initiation of myocyte differentiation enhances exogenously transplanted ADSCs persistence in vivo, and consequent tissueneovascularization, to improve cardiac function [58]. Cardiac myogenesis with fat stem cells

which have the ability to alter to myocardial cells were done not only in vitro but also in vivo. In myocardial infarcts, it was shown that these cells both alter to myocardial and endothelial cells and they decrease infarct scale. However, several problems may occur during transplantation. It becomes rather difficult for fat stem cells which are very similar to stem cells to survive after implantation. Thus, scientists have been searching new way to eliminate this problem. For instance, they have tried to use ADSCs by combining them with hemooxygenase-1 transduction. However, BMSCs and ESCs are more advantageous at this. Recent experiments are yet at animal level, but it is for certain to have human applications in the near future.

Clinical applications of MSCs are emphasized due to their potential for multiple alteration. They can especially provide tissue regeneration in cases of bone damage, heart attack, and nervous system damages. A group of researchers proved that these cells adapted to xenogeneic bone material as well. Moreover, studies have reported that MSCs which have the ability to repair defects and

damages can eliminate cartilage damage without induction and that they trigger osteogenesis in osteonecrotic tissue whose vascular microstructure has been damaged.

It has been determined that if ADSCs are cultured with β-mercaptoethanol, they have a tendency to shape into neuron-like cells and they express early markers of neuronal line, neuronal nuclei and neuro-specific enolase. However, mature neuron markers could not be found during long-term culturing. This may be due to the restrictions of the used mediums. Recent studies show that peripheral nerve damage is repaired by ADSCs. In animal models, subjects who were given peripheral nerve damage via cell transplantation have shown functional recovery. On the other hand, it was reported that they can induce to Schwann-like cells and repair myelination damage. Although their mechanisms have not been fully understood, it is commonly believed that these mechanisms are related to paracrine secretion.

In order to become different cells in the body, stem cells require growth factors. Growth factors provide the necessary nutrition for stem cells to grow and form new

cells. Factors that play an important role in the repairment process when there is any sort of damage and facilitate paracrine mechanism in tissue growth are released from the cells. Paracrine factors include growth factors and citokynes. Their functions are to enable blood flow to ischemic tissues, to prevent apoptosis, to regulate inflammation, and to facilitate regeneration of damaged tissue. Studies show that VEGF and HGF are important paracrine factor and that angiogenesis and arteriogenesis are regulated by these enzymes. Moreover, it has been reported that VEGF and HGF levels in the tissue increase when MSCs are used in treatment of heart attacks. In the studies on human adipose-derived stem cells, it has been shown that Rehmania glutinoa oligosaccharide increases proliferation capacity and cell vitality by enabling the release of paracrine VEGF and HGF.

ADSCs are key regulators of new blood vessel formation and widely investigated for their role in tissue regeneration and tumorigenesis. However, the cellular and molecular mechanisms through which ADSCs regulate angiogenesis are not well understood. Here, it was our goal

to test the functional contribution of ADSC-mediated extracellular matrix (ECM) remodeling on endothelial cell invasion. To isolate the effect of ECM-remodeling, ADSCs were embedded within 3-D collagen type I hydrogels and pre-cultured for 7 days; controls were not pre-cultured. A confluent monolayer of human umbilical vein endothelial cells (HUVECs) was seeded on top and its invasion into the underlying hydrogel was analyzed. Without pre-culture, ADSCs inhibited vascular sprouting by stabilizing the endothelium. In contrast, 7 day pre-culture of ADSCs drastically increased invasion by HUVECs. This effect was largely mediated by proteolytic ECM degradation by ADSC-derived matrix metalloproteinases (MMPs) rather than vascular endothelial growth factor (VEGF), as our results indicated that blockade of MMPs, but not VEGF, inhibited endothelial sprouting. Collectively, these data suggest that the angiogenic capability of ADSCs is modulated by their proteolytic remodeling of the ECM, opening new avenues for pro- and anti-angiogenic therapies [59]. It is known that many cytokines related to angiogenesis are released from ADSCs. Most frequently seen ones are TGF-β and VEGF.

It is reported that they inhibit inflammation of TGF-β in xenogenic bone materials. Moreover, they increase the growth of TGF-β fibroblast, osteoblast and Shwann cells. It was reported that they are significant in providing the revascularization of TGF-β, bFGF, and VEGF expressions through cell transplantation. All of these show that ADSCs alter to vascular endothelial cells in ischemic or necrotic damages or they help revascularization directly through paracrine secretion. Thus, the area with lesion can get more nutrition via revascularization, and this shows that a nutrition source is needed for tissue regeneration. However, although ADSCs moves are not yet fully understood, there is an assumption that micro-frame can be changed by stimulating the secretion of such factors as VEGF and TGF-β. Moreover, it is thought that VEGF and TGF-β secretion can be achieved from ASCs through their activation.

It has been reported that diet increases the incident of many diseases such as prostate cancer and hepatic cancer. This makes ASCs suspect, because in some studies it was observed that ASCs in the fat tissue which was formed due

to overeating surprisingly strengthen prostate cancer cells by increasing tumor vascularization via FGF-2. In later studies, it was found out that ASCs eliminate or reduce the latent period of tumor cells that promote melanoma growth, and that exosomes released from tumor cells turn ASCs into myofibroblastic phenotype by activating intercellular signal pathway. All these negative aspects notwithstanding, it was also found out that ASCs can inhibit tumors in many different ways. Because of ASCs surprisingly capacity for migrating against malignant glioma, there are studies in order to enable them to play a therapeutic role in carrying the oncoliytic myxoma virus in vitro and in vivo. Moreover, these cells not only help carry the virus, but they also enable the replication of it. Thus, these replications are promising in shedding light on the treatment of malignant glioma. Additionally, it was reported that ASCs inhibit the growth of pancreatic ductal adenocarcinoma and stop the cellular cycle of tumor cells.

In another study, animals with urinary incontinence were transplanted ADSC isolating it. Before and after the transplantation, urethra tissue and tissue around urethra

were subjected to urodynamics test and morphological evaluation. Urodynamics measures were done when bladder was full, and pressure value was measured. It was observed that in animals that were given ASC local urethral muscle was stronger and the sphincter improved both morphologically and functionally. Although a spectrum of options is available for erectile dysfunction (ED) treatment, ED in diabetics, postprostatectomy patients, and those with Peyronie's disease (PD) may be more severe in degree and less likely to respond to conventional medical therapies. One of the more fascinating strategies in preclinical development to treat ED is stem cell transplantation. Depending on the cell type, recent research has demonstrated that with transplantation, these stem cells can exert a paracrine effect on surrounding penile tissues and differentiate into smooth muscle, endothelium, and neurons. The preclinical works using animal models for the various disease processes responsible for ED have provided evidence supporting stem cell differentiation and cavernosal tissue incorporation [60]. An other application of ADSCs has produced positive effects on erectile function in various

animal models of erectile dysfunction. In acute animal models, such as cavernous nerve injury-induced erectile dysfunction and chemically induced Peyronie's disease, engraftment and differentiation have not been observed, and stem cells are believed to interact with the host tissue in a paracrine fashion, whereas in chronic disease models some evidence suggests both engraftment and paracrine factors may support improved function. Clinical trials are now investigating therapeutic efficacy of cellular therapy, whereas the first safety studies in humans have recently been published [61].

In standard approaches, pure tissue reconstruction is done with autologous tissue flaps, fat transplantation, and alloplastic implants. All these approaches prove to be at a disadvantage in terms of implant migration, absorption, being foreign to the body, and donor-site morbidity. ASCs are used in many fields, especially in plastic surgery, and they resolve such limitations. Autologous free fat transfers have many applications in plastic surgery. They can be used in reconstruction and breast reshaping without oncologic resection. They can be used effectively in HIV

lipodystrophy, face reshaping, hand and face rejuvenation operations. Moreover, fat tissue is used in correcting the asymmetrical structure in Poland Syndrome and Parry-Romberg syndrome, and in repairing defects resulting from hip fractures. Their appropriateness for vascular tissue and valve transplants. Recently, they have begun to be used in orthopedic surgeries, laryngology, nerve surgeries, general surgeries, and vascular surgeries.

Periodontal tissue regeneration with ASCs has been reported in experimental animal models. The interaction between ASC's and PRP(Platelet- rich plasma) promotes tissue regeneration and neovascularization. Tobita et al studied the combination of ASC's and PRP with the later used as a cell vehicle/carrier in animal models [62]. In a study using rat periodontal tissue defect model, green fluorescent protein labeled rat ASC's were implanted with PRP and evaluated 2,4,and 8 weeks after implantation [63].

Advances in stem cell biology have yielded promising results in vitro and in vivo suggesting that future applications in regeneration may be achievable. Because

large quantities of adipose derived stem cells can be harvested from adipose tissue, it might be possible for clinical use. The ability of the ASCs to differentiate into several tissues makes it particularly attractive type of adult stem cell for periodontal regeneration and tissue engineering. The periodontal microenvironment may induce ASC to grow and differentiate into periodontal tissues and the ASCs themselves might secrete various cytokines that stimulate resident progenitor cells. Further preclinical and clinical studies are needed to determine whether ASC based therapies can fulfill expectations and can be used successfully in periodontal regeneration [64]. In addition to this, it was also seen that salivary glands damaged due to radiation exposure are partly regenerated, that radiation is gone and blood flow is increased after ADSC transplantation. Except for HSC, among the adult KH varieties, the others do not currently have a routine clinical application area. HSCs have been used successfully for many years in the treatment of many genetic-based blood diseases, especially bone marrow-derived cancers. However, there are many ongoing and finished clinical trials and research studies with neural and

cardiac SSCs, especially MSCs. These studies continue in plastic surgery, orthopedics, cardiovascular diseases, autoimmune diseases, neuromuscular / neurodegenerative diseases and some metabolic diseases. Permanent neurological deficits may develop due to neuron and/or axonal damage in these diseases in CNS, which are relatively resistant to regeneration. CH transplantation is to provide trophic contributions to stalled tissue healing, to ensure myelin repair, and most importantly, to repair the damage by stimulating endogenous CHs. Recent studies have shown the presence of endogenous CH in the CNS. The idea that CH treatment can provide neurological and thus functional recovery by activating these cells is becoming widespread. Limited neurogenesis, the presence of active inhibitors that delay healing, and the inhibition of glial scar tissue seem to be known obstacles to the treatment of CH in CNS diseases. However, KH technology, which has passed through a period of living cloning from somatic cells (SH), develops different and unique new resources in neurosurgery. Supporting these treatments with cellular treatments provides more positive scientific results.

## STEM CELL THERAPY IN NEUROSURGERY AND ITS DEVELOPMENT IN OUR COUNTRY

Before KH studies were conducted, it was thought that living things did not have the ability to regenerate in the CNS. This idea has gained another dimension with the demonstration of the existence of multipotent neuronal SSCs that can differentiate into neurons, astrocytes or oligodendrocytes under appropriate conditions. Current information is that endogenous neuronal CHs are present in the CNS and are activated in conditions such as ischemia, trauma, and degeneration. Consensus on the treatment and applications of CH in CNS has not yet developed. Recently, it has been recommended that CHs be given together with endogenous mediators and cytokines (exosomes). There are studies suggesting that it can be surgically transplanted to the damaged area, given lumbar puncture (LP), intravenous (IV), intramuscular (IM) or combined. Pendharkar et al. of neuronal CHs in hypoxic ischemic encephalopathy (HIE), Nakamizo et al. reported that bone marrow-derived CHs have therapeutic properties when given IV in glioma. In their study, they emphasized that giving IV CH treatment is a minimally

invasive method. In their research, they showed that although IV administered CHs remained in the systemic circulation to a large extent, they settled in the CNS. Callera and Melo, on the other hand, performed LP and CD34 magnetically labeled autologous bone marrow CH transplantation to patients with chronic spinal cord injury (SCI). In their study, they showed the patients that the marked CHs clustered in the damaged area in the post-procedure control whole spinal cord Magnetic Resonance Imaging (MRI). Marconi et al. They reported that human adipose tissue-derived mesenchymal CH treatment applied as IM positively affects peripheral nerve regeneration in a rat model with sciatic nerve damage. Based on this study, they concluded that IM CH transplantation may also have a positive effect in patients who develop spasticity or atrophy in their extremities due to CNS disease. Regional CH transplantation is applied surgically in SS. It has been stated that CH transplantation can be performed with the help of microsurgery or stereotaxy, especially in areas where syrinx develops in the lesion area. Topcu performed intrahippocampal CH transplantation in an experimental epilepsy model and

stated that this method could be an alternative for the treatment of resistant temporal lobe epilepsy. It has been shown that the scaffold produced with the help of bioengineering can be applied in neurosurgery practice. There are studies showing that the treatment is more successful when CH and its mediators are placed within the scaffold to the damaged area. These resorbable structures allow STSs to continue their existence and show their effects here. The scaffold does not produce a mass effect as they are absorbed after the cells are transplanted. Scaffolds can be autologous (Self-assembling peptide (SAP)) or synthetic (poly-lactic-co-glycolic acid (PLGA)). There are a number of technical problems in the creation of scaffolding. The most important of these is the risk of immune response in PLGA, but the production cost is lower. In SAP, on the other hand, while there is no immune response risk, it is more difficult to obtain technically and production cost (19,29,44). First of all, a suitable laboratory environment (GMP) is required for KH. Without GMP conditions, it is impossible to produce cells and transplant them into humans (46). Currently, there are 2-3 centers in Turkey that provide this

environment. However, there are quite a few that are under construction. In our country, this treatment T.C. It is carried out with the permission of the Ministry of Health and clinical research applications are made for individual or Phase 2 studies within the scope of clinical trials for each patient.

Stem cell researches are promising in the treatment of stress urinary incontinence, although they are developing rapidly. While mesenchymal stem cells derived from bone marrow and fat cells are used more in murine transplants, mesenchymal stem cells derived from muscle tissue are used more in human studies. However, the ideal group has not been determined yet. Transplantation pathways of cells vary. These are intraurethral, periurethral and intravenous administrations. Another point that differs is the mechanisms of urinary incontinence in animals. These are sciatic or pudendal nerve incision, urethral cryo or chemical injury, and vaginal distention method. It is therefore difficult to make an exact comparison. When studies were examined based on similar methodologies, Xu et al. in 2010 and Kim et al. in 2011 created a urinary

incontinence model by inducing pudendal nerve damage on rats. In the examinations, improvement was found in leak point pressures after urethral bone marrow-derived stem cell injection in rats. In a similar study, Kinebuchi et al., this time urethral damage was achieved in rats by injection of a toxic substance, and then bone marrow-derived stem cell transfer was applied. Researchers have shown improvement in leakage point pressure in this study. However, no statistically significant difference was observed when compared with the control group. It should be kept in mind that the incontinence model created in this study is much more serious and common. Again, after histological examination, Kinebuchi et al. found a higher rate of striated muscle tissue in the transplantation group than in the control group. Apart from bone marrow-derived stem cell transplants, fat-derived mesenchymal cells have also been used in some studies and a urinary incontinence model has been created with different methods. Lin et al. performed a fat-derived mesenchymal cell transfer from animals to animals after they created a urinary incontinence model with vaginal expansion method on rodents. In their subsequent investigations, a

decrease in voiding dysfunction was detected in stem cell transplanted animals (33% vs 80% in the control group). As a result of the successful results in animal studies, the researchers conducted human studies on stem cell applications in urinary incontinence. Lee et al. evaluated the results after transferring stem cells obtained from cord blood transurethrally to 39 patients with stress urinary incontinence. In the examinations, it was determined that 29 of 39 patients had a subjective improvement of 50%. In a more recent study, 12 female patients with stress urinary incontinence and fixed urethra were included in the study, and stem cells obtained by deltoid muscle biopsy were transferred to the patients. After the cells were transferred with a single injection under endoscopy, the patients were examined. During the 12-month follow-up, the pad test was found to be negative in 3 patients, and while there was a positive improvement in the pad tests of 7 patients, no improvement was observed in their voiding functions. Incontinence progressed worse in 2 patients. No side effects were observed in human studies, and the most important limiting factor is the small number of patients and the absence of a control group. As mentioned before,

there are many opinions and theories about how stem cells work in the treatment of stress urinary incontinence. The possibilities are that it is the result of direct cell integration, mass effect, trophic effect, or immune system interference. In the studies performed by Lin et al., stem cells were labeled with GFP. Cell differentiation and successful fusion of the detected cells were not observed in the histological examinations performed 4 weeks after the transplantation of the fat-derived cells. However, the positive changes encountered while evaluating the results suggest that they are most likely due to trophic effects, not direct stem cell muscle regeneration. In other studies, it is supported that the effect is mostly due to the mass effect. In their studies, Kim and Xu found that there was an increase in muscle mass in the rat urethra after transplantation. Confidence regression was achieved with an obstructive effect rather than a functional one. In conclusion, although stem cell transplantation is a useful treatment method in urinary incontinence models, there are still question marks about its mechanism of action.

## Anal Incontinence

Anal incontinence is encountered in approximately 2% to 15% of the general female population. Anal incontinence often occurs as a result of sphincter injury during labor. Approximately 0.7% to 9.3% of all labors can cause anal sphincter injuries. Although sphincter injuries are detected and repaired after birth, sphincter damage can continue in 50% of them. Anal sphincter injuries occur as a result of muscle rupture, incorrect sphincter repair, and long-term sphincter hypaxia. Unfortunately, after primary or secondary sphincter repair, only 30% of patients achieve continence in their 5-year follow-up after surgical treatment[20,2l]. Myoblasts, muscle-derived stem cells and mesenchymal stem cells show that good results can be obtained in the treatment of anal incontinence. In the studies by Lane et al., it was observed that muscle-derived stem cells labeled with GFP fuse with anal sphincter muscle cells after transplantation and continue their life. Today, stem cell applications in the treatment of anal incontinence are mostly based on animal studies, and only one human study has been reported so far. In this study,

muscle-derived stem cells were used in women with anal sphincter injury as a result of birth trauma. The women included in the study were followed for 1 year, and the first results revealed that the use of stem cells was positive and safe. Wexner Incontinence Scale, anal squeezing pressure and quality of life scales were used when patients were evaluated 1 year after treatment. In this study, myoblasts obtained as a result of autologous pectoral muscle biopsy were used. As a result, at the end of the 12th month, an average of 13% decrease was observed in the Wexner Incontinence Scale scoring, while there was no change in anal squeezing pressures, and an average increase of 30 points was observed in the quality of life of the patients. Despite the absence of a control group and a small group of patients in this study, it can be concluded that autologous myoblast transfer is a safe, well-tolerated option and may improve anal incontinence symptoms. Of course, longer follow-up times with larger patient groups are required for results to be more meaningful. However, when the results obtained as a result of all animal experiments and human studies are evaluated, the use of

stem cells in the treatment of anal incontinence is promising.

**Stem cells in the treatment of infertility**

Degenerative diseases of specialized cells are among the disease groups for which modern medicine is helpless. Reproductive cells are also highly specialized cell groups. Germ cells are irreversibly lost after degenerative diseases of both male and female reproductive organs (such as premature ovarian failure, post-varicocele or azoospermia developing due to various environmental reasons) or due to various congenital diseases. In such cases, assisted reproductive technologies that we currently use are useless. At this point, obtaining reproductive cells with stem cell treatments appears as an alternative. In vitro fertilization is the most important form of treatment in infertility treatment in recent years. Especially in patients who do not have gametes or gonads, egg donation is the only option. For this reason, many researchers have sought alternative treatment methods. The first of these is the artificial creation of gametes. In recent years, nuclear transplant (NT) embryonic or adult stem cell technology

has led to a mountain of new alternatives. For this purpose, somatic cell haploidization, which is the process of forming gametes from somatic cells or embryonic cells, has been defined. This process is forcing somatic cells to divide by meiosis, and as a result, cells with a haploid number of chromosomes are formed. However, although it is a successful definition in theory, the formation of gametes from somatic cells in practice has not been reported yet. It is a generally accepted theory that in women, germ cells are present in a certain number in the gonads at birth and decrease through atresia throughout life and are depleted at the end of the reproductive period. Alien et al. questioned this theory and showed newly formed oocytes in the ovarian cortex of rats. Others later presented similar data, but the sample sizes of these studies are very small and their results are questionable. In a study, they suggested that oocytes are formed from hypotensive progenitor cells in the outer part of the cortex in the human ovary. From animal experiments, it has been observed that the number of oocytes in the ovaries remains stable despite significant loss of oocytes. In this case, neo-oogenesis must occur to maintain the normal fertile period.

Again, donor-derived ovulated oocytes have been shown in some patients after bone marrow transplant. It has been shown in vivo that embryonal stem cells (ESC) originating from the inner cell mass of the blastocyst can differentiate into three germ sheets, germ cells and trophectodermal layer cells. However, such a totipotent potential was only observed when these cells were introduced into blastocysts or early embryos. Although these cells can differentiate into various cell types from the three germ sheets in vitro, they have not been shown to transform into trophectodermal and germ cells. Recent studies have shown that mouse ESCs transform into germ cells that form gametes in vitro. While some of these cells entered meiosis and supported fertilization, some of them entered partonogenesis and formed structures expressing blastocyst-like trophectoderm markers. Human ESCs cultured in vitro also expressed germ cell-specific markers. In studies conducted to date, high degeneration rates and rapid partonogenesis of oocytes obtained from in vitro animal models are the main problems. It has been shown by Dyce et al. that not only ESCs but also adult stem cells can transform into oocyte-like cells. It has been

shown that oocytes are formed from stem cells from bone marrow and peripheral blood in mice. After obtaining stable oocytes, these oocytes should be fertilized and the conceptus obtained should be able to show a healthy development. There are also various question marks about the sperm obtained from ESC. Toyooka et al. demonstrated in vitro sperm formation from ESCs. Geijsen et al. also showed that blastocyst can be formed by intracytoplasmic sperm injection method. However, it has not been revealed whether this blastocyst is normal and whether it can develop normally. In addition, the epigenic status of these gametes obtained in vitro is not clear. It is unclear whether they normally undergo meiosis and carry the correct maternal and paternal genetic makeup. Currently, germ cells derived from stem cells have not been shown to enter meiosis. Another problem related to obtaining germ cells is that germ cells obtained from ESC cannot be obtained purely and other cell types are found in culture medium. As a result, it seems possible to generate germ cells in vitro, but many related problems still await solutions. In the light of this information, new experiments should be designed and the obtained data

should be combined with techniques such as nucleus transfer from somatic cells and in vitro embryo culture.

## Stem Cell in Gynecological Oncology

Fighting cancer maintains its importance as one of the most important problems of today's medicine. Linecological cancers are among the most common diseases that threaten women's health. Despite the increase in early diagnosis and treatment opportunities with the screening programs developed today and the ease of access to health services, the diagnosis of the disease in the advanced stage poses a problem, especially in ovarian cancers. Epithelial ovarian cancers are ranked 4th for cancer-related deaths in the United States, and 1st for gynecological cancer-related deaths. is in line. The most important problems encountered in the treatment of ovarian cancers are recurrence and chemoresistance. While 80% of the patients respond to the combination of surgery and chemotherapy, unfortunately, 60-80% of the patients experience recurrence between 6 months and 2 years. In recurrent disease, the response to treatment decreases to 15%.

## Conclusion

ASCs are under investigation for a variety of therapeutic applications. These cells are known to home to some tissues such as injured tissue. Although the mechanisms underlying the migration of ASCs remain to be determined, clarification of the roles of chemokine receptors and adhesion molecules on ASCs may lead to the development of therapeutic strategies to enhance the recruitment of cultured ASCs to injured or damaged tissue.

Because human adipose tissue is a promising alternative source of stem cells, autologous ASCs will lead to novel clinical applications in various medical fields. However, a greater understanding of the mechanisms of interactions among ASCs, growth factors, and biomaterials on tissue regeneration is needed to advance the clinical utility of this therapy. Because chemokines derived from ASCs may also affect cancer metastasis or invasion, additional findings are necessary to address the safety of ASCs in the field of clinical tissue regeneration.

The adipose tissue microenvironment may induce ADSC to grow and differentiate into adipose tissues and the ADSCs themselves might secrete various cytokines that stimulate resident progenitor cells. Further preclinical and clinical studies are needed to determine whether ADSC based therapies can fulfill expectations and can be used successfully in tissue regeneration.

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