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Article review: Stem cells a healing effect in cancer therapy

Talib Fadhil Abbas Al-Zayadi [1]

Abstract

Cancer is the DNA replications mistakes, where it's enhanced by many chemical factors, environmental factors, radiation, in addition to, the viral and immunological diseases. It has been diagnosed with many types of cancers in human being, called by the organs and classified to breast cancer, lung cancer, prostate cancer, and colon cancer.etc. Oncology is the science of genetic materials disease after birth in humans, concern with all metabolic steps guide to abnormal DNA multiplications within somatic cells In order to stop these abnormal multiplications, the researchers tend to find genetic drugs within telomerase line of research; chemical drugs used to kill the cancer cells at early stages of disease; immunological drugs that used in immune system stimulation materials.

Key words: Stem cells, Regenerations, Cancer, Drug

* Correspondence author: talib.abbas@aol.com
Talib Fadhil Abbas Al-Zayadi
Collage of Pharmacy, University of Al-Muthana

Introduction

Presently, cancer therapy has entered in to an exciting new era, with traditional therapies such as chemotherapy, radiotherapy, and surgery on one side, while the stem cells on the other. Apart from another well-known role in immune-reconstitution, the stem cells have attracted much attention especially with the new gene technologies, such as the gene incorporation into the eukaryotic cells. allowing more focused delivery of the anti-cancer agents. Stem cells are reprogrammed cells which were able to develop into many different types of functioning cells, including liver, bone, and nerve cells. Stem cell-based treatments are being used and investigated for conditions as Parkinson's disease, neural degeneration following brain injury, cardiovascular disease and autoimmune disease[1,2,3]. Since 2007 there have been several new developments in the field of stem cell research that significantly change the landscape. This includes the development of induced Pluripotent Stem cells by introduction of a limited number of genes into adult somatic cells, paving the way for the generation of histocompatible or patient- specific pluripotent stem cells. Also,

progress has been made in growing stem cells without xenogeneic feeder cells. However, significant challenges remain. with respect to characterizing the cell product for therapy for its purity, safety, and potency in an expeditious and cost-effective manner. Some advances have been made towards understanding the basic biology of stemness and their differentiation into different cell lineages, but harnessing of their promised potential to usher in the era of regenerative medicine is still a long way to go. Several clinical trials have been carried out using autologous or allogenic CD34+ve hematopoietic stem cells or mesenchymal stem cells (MSCs) in a variety of clinical indications but most of these have been Phase I or early Phase II trials[4,5,6.]

Stem Cell Types and sources

Stem cells are unspecialized cells that have two defining properties, the ability to differentiate into other cells and the availability to self-regenerate. Self-regeneration is the ability of stem cells to divide and produce more stem cells. During early development, the cell division is symmetrical, each cell divides to give rise to daughter cells each with the same potential. Later in development, the cell divides asymmetrically with one of the daughter cells produce also a stem cell and the other a more differentiated cells[7,8].

Embryonic stem cells

Embryonic stem cells (ESCs) are derived from the cells of the inner cell mass of the blastocyst during embryonic development. ESCs have the capacity to differentiate into any cell type and the ability to self-replicate for numerous generations. Germinal stem cells are derived from primarily germinal layers of the embryo. They differentiate into progenitor cells to produce specific organ cells. Somatic/adult stem cells are progenitor cells as they are less totipotent i.e. less replicative life span than ESCs. They exist in mature tissue such as hematopoietic, neural, gastro-intestinal and mesenchymal tissues[10,11].

Adult stem cells

Sources of adult stem cells include the umbilical cord, amniotic fluid, bone marrow, adipose tissue, brain, and teeth. Adult stem cells are not subject to the ethical controversy that is associated with embryonic stem cells; they can also be autologous and isolated from the patient being treated, whereas embryonic stem cells cannot. Immunohistochemical study of expression marker CD34 in various studied groups

show that chorion application affected on CD34 in the pulp. While amniotic fluid affected on dental follicle[12].

Multipotent stem cells

Stem cells could be dividing into three main categories: embryonic, germinal and somatic. Embryonic stem cell (ESCs) originates from the inner cell mass of the blastocyte. ESCs are omnipotent and have an indefinite replicative life span, which is attributable to their telomerase expression[9] reports have cautioned that any carcinogenic potential of iPS cells should be fully investigated before any commercialization can be realized. The micro RNAs expression has been reported as a requisite to bypass G1/S checkpoint, thus for the self-renewal characteristic of stem cells[13].

Induced pluripotent stem cells (iPS)

The newly discovered iPS cells are adult or somatic stem cells that have been coaxed to behave like embryonic stem cells .iPS cells have the capacity to generate a large number of stem cells as an autologous source that can be used to regenerate patient-specific tissues. However, even the authors of these recent reports have cautioned that any carcinogenic potential of iPS cells should be fully investigated before any commercialization can be realized. The micro RNAs expression has been reported as a requisite to bypass G1/S checkpoint, thus for the self-renewal characteristic of stem cells[13].

Umbilical cord blood stem cells (UCBSCs)

UCBSCs derive from the blood of the umbilical cord. There is a growing interest in their capacity for self-replication and multilineage differentiation, and UCBSCs have been differentiated into several cell types that resemble cells of the liver, skeletal muscle, neural tissue, pancreatic cells, immune cells, and mesenchymal stem cells. Several studies have shown the differentiation potential of human UCBSCs in treating cardiac and diabetic diseases in mice. The greatest disadvantage of UCBSCs is that there is only one opportunity to harvest them from the umbilical cord at the time of birth. Similarly, amniotic stem cells can be sourced only from amniotic fluid and are therefore subject to time constraints[14].

Adipose-derived stem cells (ASCs)

It has been shown that there is no significant difference in the cell growth kinetics, gene transduction of adherent stromal cells and yield from stem cells obtained from bone marrow or adipose tissues. ASCs are typically isolated from lipectomy of liposuction aspirates. They have been differentiated into adipocytes, chondrocytes, myocytes, and neuronal and osteoblast lineages, and may provide hematopoietic support [16,18].

Bone marrow-derived stem cells (BMSCs)

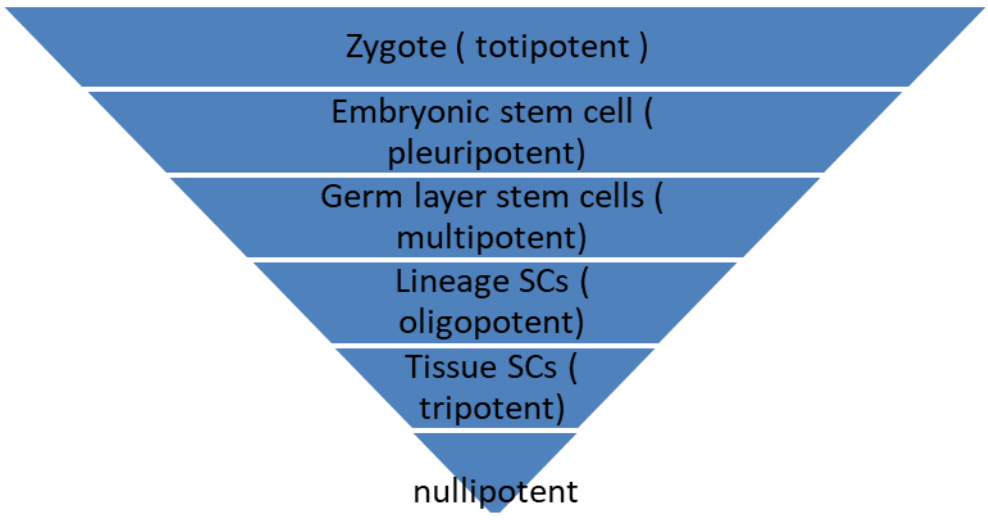
Most commonly ASCs are required from the bone marrow and peripheral blood. The Bone marrow (BM) aspiration is one of the common procedures performed to obtain ASCs, but it's associated with morbidity in the form of wound infection and sepsis complication. BMSCs consist of both hematopoietic stem cells that generate all types of blood cells and stromal cells (mesenchymal stem cells) that generate bone, cartilage, other connective tissues, and fat[15,50].

Table 1: Differential potential ranges from totipotential stem cell to Unipotentail cells/

Differentiation potential	Number of cell type	stem cell	Cell resulting from differentiation
Totipotential	all	Zygote	All cell types
Pleuripotential	all	Cultured human ES	Cells from all 3 germ layers
Multipotential	Many	Hematopoietic cell	Skeletal muscle, cardiac muscle, liver cells, all blood cells
Oligopotential	few	Myeloid precursor	5 types of blood cells
Quadripotential	4	Mesenchymal progenitor cell	Cartilage cell, stromal cell, bone forming cells
Tripotential	3	Glial- restricted precursor	Astrocytes, oligodendrites
Bipotential	2	Bipotential precursor	B cell macrophages
Unipotential	1	Mast cell precursor	Mast cells

Stem cell in development

The zygote is the ultimate stem cell. It's totipotent with the ability to produce all the cell types of species including the trophoblast and embryonic membranes. Development begins when the zygote undergoes several successive cell divisions, each resulting in a doubling of the cell number and a reduction in the cell size. At 32-64 cell stage, each cell is called blastomere1. The blastomeres are stick together to form a tight ball of cells called a morula. Each of these cells retains totipotent. Trophoblast cells along the periphery develop into the embryonic membranes and placenta while in the inner cell mass develops into the fetus. Beyond the blastocyst stage, development is characterized by cell migration in addition to cell division. The gastrula is composed of three germ layers: ectoderm, mesoderm, and endoderm. Early in embryogenesis, some cell migrates to the primitive gonad or genital ridge. These are the precursors to the gonad of the organism and are called germinal cells. These cells are not derived from any of the three germ layers but appear to be set aside. Stem cells in late development, there is a loss of potential and again of specialization, a process called determination. The cells of the germ layers are more specialized than the fertilized egg or the blastomere. The germ layer stem cells give rise to progenitor cells[17].



Pyramidal smart art of stem cells during differentiation (transit-amplifying cells). http://stemcells.nih.gov/info/scireport/chapter4.asp

Role of progenitor cells in development

While there is consensus in the literature that a progenitor is a partially specialized type of stem cells, there are differences in how progenitor cell division is described. For instance, according to sources, when a stem cell divides at least one of the daughter cells it produces is also a stem cell, when a progenitor cell undergoes cell division it produces two specialized cells. Otherwise, explain that a progenitor cell undergoes asymmetrical cell division, while a stem cell undergoes symmetrical cell division. The apparent inconsistency of these two versions illustrates the diversity and complexity of progenitor cells and their role in differentiation. This diversity is reflected in the nomenclature as well progenitor cells are also transit-amplifying cells, precursor cells, progenitor, lineage stem cells, and tissue determined stem cells[19,20].

Role of adult stem cells in tissue repair

During development, stem cells divide and produce more specialized cells. Stem cells are also present in the adult in far lesser numbers. The role of adult stem cells (somatic stem cells) is believed to be replacement of damage and injured tissue, observed in continually, replenished cells such as blood cells and skin cells. Stem cells have recently been found in other tissues, such as neural tissue. Organ regeneration has long been believed to be through organ-specific and tissue stem cells. Hematopoietic stem cells were believed to replenish blood cells, stem cell of the gut to replace cells of gut and so on. Recently, using cell lineage tracking, stem cells from one organ have been discovered that divide to form cells of another organ. Hematopoietic stem cell can give rise to the liver, brain, and kidney cells. This plasticity of adult stem cells have been observed not only under experimental conditions but also in people who have received bone marrow transplants[21]. Tissue regeneration is achieved by two mechanisms:

1- Circulating stem cells divide and differentiate under appropriate signaling by cytokines and growth factors, e.g. blood cells.

2- Differentiated cells which are capable of division can also self-repair e.g. Hepatocytes. Endothelial cells, smooth muscle cells, keratinocytes, and fibroblasts. These fully differentiated cells are limited to local wound healing in the skin, epidermal stem cells and bone-repair 4. Marrow progenitor cells both contribute to the processes of repair. Thus it is likely that organ-specific progenitors and hematopoietic stem cells are involved in the repair, even for another organ repair[3].

Role of stem cells in cancer therapy

Ontogeny (development of an organism) and Oncology (cancer development) share many common features. From the 1870s the connection between development and cancer has been reported from Shanghai centers of cancer therapy [22]. Existence of cancer stem cells with aberrant cell division has also been reported more recently. The connection between cancer and development is clearly evident in the teratocarcinoma. As early as 1862, Virchow discovered that the germ in cell tumor teratocarcinoma is made up of embryonic cells. In the 1970s, Stevens derived embryonic carcinoma cells from teratocarcinoma[23,24]. Pyramidal smart art of stem and progenitor cells differentiates and produces different sublineages of cells, resulting from response to varied growth factors. Malignancies of hematopoietic system originate from two sources: Those with increased growth in an early stem cell produce acute leukemia; while those that arise from a decreased response to death or differentiation in a stem cell produce chronleukemia. Cancer is the most common cause of mortality and morbidity in UK[3.] Despite recent advances in the treatments of cancer, the clinical outcome is yet far away from expectation. Use of the stem cell in immune-modulation or reconstitution is one of the methods used for decades in cancer therapy. Stem cells have self-renewal capacity with highly replicative potential in multilineage differentiation capacity[5]. It is unlikely that all the mutations could occur in the lifespan of the progenitor/ mature cell. Therefore, cancer stem cells should be derived from either the self-renewing normal stem cells viability [25,26,27 .]

The choice of source of stem cells for cancer therapy

Ideally, Embryonic stem cells (ESCs) are primitive (undifferentiated) cells derived from a 5- days pre-implantation embryo that are capable of dividing without differentiating for a prolonged period in culture, known to develop into cells and tissues of the three primary germ layers. The purposes of using this type of stem cells are due to higher totipotency and indefinite life span compared to ASCs with lower totipotency and restricted life span. However, the use of ESCs has ethical constraints[3]. The stem cells with higher totipotency have been shown to be more tumorogenic in mice[28,29]. Thus, for ease of availability and lesser constrained on ethical issue, ASCs are the stem cells most commonly used for research and therapeutic purpose. The other reason for the use of ASCs is their easy accessibility compared to ESCs in the bone marrow and have the ability to differentiate both in vivo and in vitro into the different mesenchymal cells such as bone, cartilage, fat, muscle, tendon [30,31].

Implications for cancer treatment

At present, cancer treatment is targeted at its proliferation mechanism and its ability to metastasis. Most treatments were targeted at rapidly dividing cells, at the molecular targets that present the bulk of the tumor. This may explain the failure of treatments to eradicate the disease or the recurrence of the cancer[8]. Although current treatments can shrink the size of the tumor, these effects are transient and usually don't improve patients survival outcomes [32] . For tumors in which the cancer stem cells play a role, three possibilities exist. First, the mutation of normal stem cells or progenitor cells into cancer stem cells can lead to the development of the primary tumor. Second, during chemotherapy, most of the primary tumor cells might be destroyed but if cancer stem cells did not eradicate, they become refractory cancer stem cells may and may lead to a recurrence of the tumor. Third, the cancer stem cells may emigrate to distal sites from the primary tumor and cause metastasis[10]. Theoretically, identification of the cancer stem cells may allow the development of treatment modalities that target the cancer stem cells rather rapidly dividing cells in the cancer.

This way cure cancer as remaining cells in the cancer growth has the limited proliferative capability. If cytotoxic agents spar-tumor initiating cell (TICs) [tumor-initiating cell function, cancer stem cells] the disease is more likely to replace. The TICs have been shown to have different sensitivity to different chemotherapeutics agents such as TICs in leukemia are less sensitive to daunorubicin and cytarabin[33,34]. Although, the idea of therapies focused on the cancer stem cells may look exciting, targeting the cancer stem cells might not be easy. The cancer stem cells are relatively quiescent compared to other cancer cells and do not appear to have the hyper-proliferation signals activated such as tyrosine kinase. These make the cancer stem cells resistant to the toxicity of the anti-cancer drugs, which traditionally target the rapidly dividing cells[35]. These deregulated signaling pathways and gene expression may have an impact on the response to cancer therapy.

Stem cells in practice

Embryonic stem (ES) cells are obtained from the inner cell mass and cultured from mouse embryos since 1980s by various groups of researchers. This pioneer established murine embryonic stem cells lines that could differentiate cell types38,39. ES cell lines have been established from other mammals. Thompson and colleagues at the University of Wisconsin reported isolation of primate ES cells in 1995 and human ES cells are the best characterized of all the ES cells in 1998 42,43. Cultured stem cells have properties:

1- It is pluripotent, i.e. they have the ability to differentiate into cells derived from all three germ layers, but not the embryonic membranes.

2- ES cells are immortal i.e. cells proliferate in culture and have been maintained in culture for several hundred doublings. The advantage of maintaining stem cells in culture is that they are a source of a large number of cells in the undifferentiated state. So far other adult stem cells have not been maintained indefinitely.

3- ES cells maintain a normal karyotype generally, ES cells are maintained in culture on feeder cells (mouse fibroblast cells).

The success of maintaining hESCs begins with the propagation of healthy feeder culture. We recommend the use of mouse embryonic feeder fibroblast. Mouse fibroblast cells have grown and passage before being plated for hESCs cultures are referred to as stock fibroblasts, whereas, cells plated to support the maintenance of hESCs, and have been rendered mitotically inactive via irradiation or chemical treatment are referred to as feeder fibroblast. There have been recent reports of ES cultured on feeder cell-free medium[45]. ES cells can be induced to differentiate in vitro by culturing in suspension to form three dimensional cells aggregates called embryo bodies[46]. The cells spontaneously differentiate into various cell types, e.g. neurons, cardiomyocytes, and pancreatic Beta cells. The addition of growth factors to the culture directs differentiation to specific cell types. However, it is still challenging to isolate pure differential cell types. Teratomas are benign tumors characterized by their rapid growth in vivo and their hap hazards mixture of tissues, and, thus often have semi-semblances of organs, teeth, hair, muscle, cartilage, and even bone. In fact, it is rare that a teratoma does not contain remnants of all three germ layers. These are key characteristics of robust pluripotency and explain why teratoma formation is widely viewed in stem cell research as the "gold standard" for assessing pluripotency. Following their establishment in vivo, teratomas typically progress by remodeling their microenvironment to support their growth and the formation of the blood vessels for nutrients. Upon engraftment, teratoma formation is affected by three main factors: PSCs type. Cell number, and delivery route. For the first major factor, teratoma incidence has been shown to vary depending on the PSC type. One study observed human induced pluripotent stem cells (hiPSCs) human induced pluripotent stem cell: A type of pluripotent stem cell, similar to embryonic stem cells, formed by the introduction of certain embryonic genes into a somatic cell. Teratoma formation rate to be slower than that of human embryonic stem cells (hESCs). Human embryonic stem cells A type of pluripotent stem cells derived from early-stage human embryos, up to and including the blastocyte stage. hESCs are capable

of dividing without differentiating for a prolonged period in culture and are known to develop into cells and tissues of the three primary germ layers) due to the heterogeneity in hiPSCs gene expression levels that suggests a less stable pluripotential state in these cells[47]. Other studies demonstrate hiPSCs to be more efficient[48]. Following injection of ES cells into immunodeficient mice, teratomas develop with derivatives of all three germ layers. This is a major disadvantage of using ES cells for cell therapy since any contaminating undifferentiated cells could give rise to cancer[49] .

Adult or somatic stem cells, the existence of hematopoietic stem cells were discovered in the 1960s, followed by the discovery of a stromal cell. Only in the 1990s, the scientists did confirm the reports of neural stem cells in mammalian brains. Since the stem cells have been found in the epidermis, liver, and several other tissues[52]. Adults stem cells offer hope for cell therapy to treat diseases in the future because ethical issues do not impede their use. In addition, if the patient's own cells are used, immunological compatibility is not an issue. However, ES cells have been found to be superior for both differentiation potential and ability to divide in culture. Plasticity is a newly recognized ability of stem cells to expand their potential beyond the tissue from which they are derived. For example, dental pulp stem cells develop into the tissue of the teeth but can also develop into neural tissue[53]. Cell fusion, ES can fuse in vitro with neuronal cells and with hematopoietic stem cells [54,55]. This has started a new debate in adult stem cell plasticity, namely that some cells may have fused and the nucleus was programmed instead of transdifferentiating, which is conversion one type to another.

Methodology

Isolation of bone marrow –mesenchymal stem cells Using Primary explant culture method (direct plating)

According to the original working of Friedenstein[56] used by Malachi and collogues, by utilizing adherent cultures of untreated whole bone marrow, after preparation of bone marrow uniform cell suspen¬sion. The cell suspension was centrifuged at 1000 rpm for 5 minutes at 18ºC, Supernatant containing thrombocytes and erythrocytes was discarded and the cell pellet was resuspended with complete growth medium RPMI-1640 with 10%FBS, or Complete Dulbecco's modified Eagle medium (DMEM), high-glucose formulation, containing: 10% fetal bovine serum (FBS), heat-inactivated 1 hr at 56°C; 1% nonessential amino acids, 2 mM L-glutamine, 50 µM 2-ME 100 U/ml penicillin 100 µg/ml streptomycin sulfate.

Culturing and Expansion of BM-MSCs

The cell suspension was seeded in plastic tissue culture flasks 25cm2 with 5ml culture medium RPMI-1640 supple¬mented with 10% FBS and antibiotics at a plating density of 16 × 106 cells/ml using direct plating method. The culture was incubated at 37Cº in control medium for growth until complete confluent monolayer cell culture was reached. The MSCs were isolated based on their ability to adhere to the culture plates. After 24 hours, red blood cells and other non-adherent cells were removed and washed 2-3 with PBS then fresh medium was added to allow further growth. This feed¬ing process then lasted twice in a week until we made the first passage of MSCs.

Immunocytochemistry analysis of BM-MSCs

Immunocytochemical analysis was performed on cells grown on coverslips. A coverslip was placed inside the tissue cul¬ture dish (9cm2) to allow cells to grow over it for subsequent analysis at density 1×106 cell/ml. After the cells had grown to near 80% confluency (P3), the culture attached cells on cover¬slip were washed three times with PBS and fixed with 4% for¬malin prepared in PBS and left for 10 minutes and then dried then to step 7 of the following immunohistochemistry. Using the tissues will be within the :same steps as follow

1- Slide baking: the slides were placed in a 45° angled inclined position in a hot air oven at 60°C overnight.

2- Deparaffinization: the slides were immersed in xylene for 15 minutes two times at room temperature.

3- Rehydration: the slides were immersed sequentially in the following solutions at room temperature starting with:

Twice in absolute ethanol for 5 minutes in each concentration 95% - 90% -80% -- 70%.And in Distilled water for 5 minutes.

4- Enough drops of hydrogen peroxide block were added to slides and incubated in a humid chamber at 37ºC for 10 minutes, then socked 2 times in buffer (5minutes for each).

5- Enough drops of protein block were added to slides and incubated at 37ºC for 10 minutes. Then washed 2 times in buffer (5minutes for each), finally drained and blotted gently.

6- Diluted primary antibody was applied to each slide, incubated in humid chamber at37ºC overnight. Early in the next day, the slides were washed in buffer 4 times (5 minutes for each), finally drained and blotted gently as before.

7- Enough drops of secondary antibody (link antibody yellow drops) reagent was added and incubated in a humid chamber for 20 minutes at37ºC. After that, the slides were washed 4 times in buffer (5minutes for each), finally drained and blotted gently.

8- Streptavidin-HRP antibodies (red drop) was applied on tissue and incubated for 20minutes at37ºC. After that, the slides were washed 4 times in buffer (5minutes for each), finally drained and blotted gently.

9- Diluted DAB (3'diaminobenzidine Tetrahydrocloride) was applied on the tissue(this process was done in the darkroom) and incubated in a humid chamber for 10 minutes at37ºC. Then slides washed carefully in tap water for 5 minutes.

10- The slides were bathed in hematoxylin counterstain for 1-2 minutes then they were rinsed with tap water for 10 minutes.

11- Dehydration: the slides were dehydrated by immersing them in ethanol and xylene containing jars as follows (70% - 80% - 90% - 95%). And twice in absolute ethanol for 1 minute each.

-Xylene for 1minute

- Fresh xylene for 5 minutes

12- One to two drops of DPX mounting medium was applied to the xylene wet sections and covered with coverslips and left to dry for 30 minutes.

Antibody used in study

Monoclonal was used in the present study from scientific companies, USA or UK.
Preparation of reagents Dilution of primary antibodies was done by using sterile PBS in a concentration according to each data sheet of monoclonal antibodies. The antibody was tested with several runs as a technical control staining in order to reach the optimum positive run. CD34 was diluted into 1/50 times. Dilution of DAB solution DAB was prepared by mixing 1ml of (DAB Buffer) with 20µl of (DAB chromogen) in a dark tube, and then kept in a dark place until used.

Principles of the Test

The labeled streptavidin-biotin (LSAB) method utilizes a biotinylated secondary antibody that links primary antibodies to a streptavidin- peroxidase conjugate, and by adding the chromogen substrate, a colorimetric reaction will form at the antigen-binding site. In this method, a single primary antibody subsequently is associated with multiple peroxidase molecules, and because of the large enzyme-to antibody ratio, a considerable increase in sensitivity is achieved compared to direct peroxidase-conjugate methods. DAB (3'diaminobenzidine Tetrahydrocloride) substrate offers the greatest sensitivity in the horse-radish peroxidase enzyme system as a colorimetric chromogen; a brown precipitate will form at the antigen-binding site.

Cytometry Investigation of MSCs

MSCs in all ways were immunologically tested for surface markers of MSCs. the MSCs were first detached as of the Petri-dishes with trypsin plus EDTA after that centrifuged. Three hundred thousand cells are staining through every respective antibody. Monoclonals obtained from well known scientific companies were used as MSCs positive markers. Fluorescently-labeled MSCs were finally washed once, fixed with 1% paraformaldehyde in PBS and stored in the dark at 4°C until analysis. In smallest amount twenty thousand of MSCs are utilized to test the incidence of every cell surface marker, utilizing run of cytometry.

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