Cell Therapy In Dermatology

Essay
Submitted for Partial Fulfillment of Master degree
in Dermatology and Venereology

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2010
ربّنَا أَمَّنَّا بِمَا أَنزَلْتَ وَاتَّبَعْنَا الرَّسُولَ فَأَكْتُبْنَا مَعَ السَّاهِدِينَ.

العمران (53)
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| **1-Melanoma**         | a) Tumor cell immunization.  
                         | b) Tumor-associated antigens immunization. 
                         | c) Dendritic Cell Therapy. 
                         | d) CD4 + T cells Infusion. |
| **2-Scleroderma**      | a) MSCs. 
                         | b) Autologous HSCT. |
| **3-Scleradactyly**    | Stem Cells and Fibrinogen Solution Spray. |
| **4-Epidermolysis bullosa** | Intradermal injections of allogenic fibroblasts. |
| **5-Psoriasis**        | a) Biologics targeting T cells. 
                         | b) Anti VEGF Therapy. |
| **6- Scleromyxedema**  | Autologous PBSCT. |
| **7- Vitiligo**        | a) Grafting of cultured autologous melanocytes. 
                         | b) Transplantation of autologous melanocytes. |
| **8- Graft-versus-host disease** | a) Allogeneic HSCT. 
                         | b) MSC infusion. |
| **9-Delayed wound healing** | a) Local administration of MSCs. 
                         | b) EPC transplantation. 
                         | c) Apligraf®. |
| **10- Cicatricial Alopecia** | Wnt signaling proteins. |
| AESTHETIC APPLICATIONS | TYPE OF CELL THERAPY |
| **1-Rhytids and Photoaging** | a) Allogenic Neonatal Human Fibroblasts. 
                         | b) SKP cells. 
                         | c) ADSCs and their secretory factors. 
                         | d) Autologous PRP. |
| **2- Facial Lipoatrophy** | a) Autologous Lipoinjection. 
                         | b) CAL. |
| NEUROLOGY | TYPE OF CELL THERAPY |
| **Diseased Cerebellum** | NSCs Transplantation. |
| ORTHOPEDICS | TYPE OF CELL THERAPY |
| **1- Intervertebral Disc Degeneration** | Articular chondrocytes Transplantation. |
| **2- Frozen Shoulder** | Autologous PRP. |
| ONCOLOGY | TYPE OF CELL THERAPY |
| **1-Prostate Cancer** | DC Therapy. |
| **2- Breast Cancer** | Allogeneic Lymphocytes and Allogeneic HSCT. |
| **3- Nasopharyngeal Carcinoma** | Autologous CTLs. |
| **4- Renal cell Carcinoma** | Allogeneic DC. |
| HEMATOLOGY | TYPE OF CELL THERAPY |
| **1- Sickle cell Disease** | Autologous Pluripotent Stem Cells. |
| **2-Chronic Lymphocytic Leukemia** | Transfer of Genetically Modified T lymphocytes. |

ADSCs: Adipose Derived Stem Cells.  
CAL: Cell Assisted Lipotransfer.  
CD4: cluster of differentiation 4.  
CTL: cytolytic T lymphocytes.  
DCs: Dendritic Cells.  
EPC: Endothelial Progenitor Cells.  
HSCT: Hematopoietic stem cell transplantation.  
MSCs: mesenchymal stem cells.  
NSCs: neural stem cells.  
PBSCT: peripheral blood stem cell transplant.  
SKP: skin-derived precursor.  
VEGF: Vascular Endothelial Growth Factor.
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>ACEI</td>
<td>Angiotensin Converting Enzyme Inhibitors</td>
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<td>ACR</td>
<td>autologous cell regeneration</td>
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<tr>
<td>ADSCs</td>
<td>Adipose Derived Stem Cells</td>
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<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
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<tr>
<td>BFGF</td>
<td>Basic Fibroblast Growth Factor</td>
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<tr>
<td>BMP7</td>
<td>bone morphogenetic protein 7</td>
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<tr>
<td>BMP10</td>
<td>bone morphogenetic protein 10</td>
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<tr>
<td>BMZ</td>
<td>Basement membrane zone</td>
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<tr>
<td>CA</td>
<td>carbonic anhydrase</td>
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<tr>
<td>CAL</td>
<td>Cell Assisted Lipotransfer</td>
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<tr>
<td>CD4</td>
<td>cluster of differentiation 4</td>
</tr>
<tr>
<td>CD8</td>
<td>cluster of differentiation 8</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukemia</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>CT</td>
<td>computed tomography</td>
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<td>CTL</td>
<td>cytolytic T lymphocytes</td>
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<td>DCs</td>
<td>Dendritic Cells</td>
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<td>DEJ</td>
<td>dermal-epidermal junction</td>
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<tr>
<td>DP</td>
<td>Dermal papiller</td>
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<td>DS</td>
<td>dermal sheath</td>
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<tr>
<td>EB</td>
<td>Epidermolysis bullosa</td>
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<td>EBV</td>
<td>Epstein-Barr virus</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>EGF</td>
<td>Epidermal Growth Factor.</td>
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<tr>
<td>E/M</td>
<td>Electron microscopy.</td>
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<td>EPC</td>
<td>Endothelial Progenitor Cells.</td>
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<td>ESCs</td>
<td>Embryonic Stem Cells.</td>
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<tr>
<td>EULAR/EBMT</td>
<td>European League Against Rheumatism and European Group for Blood and Marrow Transplantation.</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration.</td>
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<tr>
<td>FMSCs</td>
<td>Fibroblast Mesenchymal Stem Cells.</td>
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<tr>
<td>GFP</td>
<td>green fluorescent protein.</td>
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<td>GIT</td>
<td>Gastrointestinal tract.</td>
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<td>GMCSF</td>
<td>Granulocyte-macrophage colony-stimulating factor.</td>
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<td>GVHD</td>
<td>Graft-versus-host disease.</td>
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<td>HDMEC</td>
<td>Human Dermal Microvascular Endothelial Cells.</td>
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<td>H2O2</td>
<td>Hydrogen peroxide.</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen.</td>
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<td>HSCs</td>
<td>Hematopoietic Stem Cells.</td>
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<td>HSCT</td>
<td>Hematopoietic stem cell transplantation.</td>
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<td>ICX-RHY</td>
<td>Intercytex.</td>
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<td>IFN g</td>
<td>Interferon gamma.</td>
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<td>Igs</td>
<td>immunoglobulins.</td>
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<tr>
<td>IL 4</td>
<td>interleukin 4.</td>
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<td>IPL</td>
<td>Intense pulsed light.</td>
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<tr>
<td>LIF</td>
<td>Leukemia Inhibitory Factor.</td>
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<tr>
<td>LMP2</td>
<td>latent membrane protein 2.</td>
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<tr>
<td>mAb</td>
<td>monoclonal antibody.</td>
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</table>
- MAGEA: Melanoma antigen family A.

- MSCs: mesenchymal stem cells.


- NP: nucleus pulposus.

- NPC: Nasopharyngeal carcinoma.

- NSAIDs: Nonsteroidal anti-inflammatory drugs.

- NSCs: neural stem cells.

- PASI: Psoriasis Area and Severity Index.

- PBS: Phosphate Buffer Saline.

- PBSCT: peripheral blood stem cell transplant.

- PCAs: Primary cicatricial alopecias.

- PDGF: Platelet Derived Growth Factor.

- PMMA: Polymethylmethacrylate.

- PNs: Purkinje Neurones.


- PUVA: psoralen UV-A.

- RCC: Renal cell carcinoma.

- RDEB: Recessive dystrophic epidermolysis bullosa.

- ROS: Reactive oxygen species.

- RT-PCR: Reverse transcription polymerase chain reaction.

- SCNT: Somatic Cell Nuclear Transfer.

- SKP: skin-derived precursor.

- SSC: Systemic sclerosis.

- SSCs: Somatic Stem Cells.

- SVF: stromal vascular fraction.
- TAA: Tumor associated antigens.
- TGF: Transforming Growth Factor.
- TKIs: tyrosine kinase inhibitors.
- TNFα: Tumor necrosis factor alpha.
- tPA: tissue Plasminogen Activator.
- TRM: transplant related mortality.
- UV: Ultra Violet.
- VEGF: Vascular Endothelial Growth Factor.
- κ: kappa.
- λ: lambda.
Introduction
Introduction

Cell therapy is the transplantation of human or animal cells to replace or repair damaged tissue and/or cells. Cell therapy is, in effect, a type of organ transplant. The procedure involves the injection of either whole fetal xenogenic (animal) cells or cell extracts from human tissue. The latter is known as autologous cell therapy if the cells are extracted from and transplanted back into the same patient. Cell therapy has been used successfully to rebuild damaged cartilage in joints, repair spinal cord injuries, strengthen a weakened immune system, treat autoimmune diseases such as Acquired Immune Deficiency Syndrome (AIDS), and to help patients with neurological disorders such as Alzheimer's disease, Parkinson's disease, and epilepsy (Martin, 2007).

Cell therapy has proven efficacy in many fields. New insights into the biology of neural stem cells (NSCs) have raised expectations for their use in the treatment of neurologic diseases. Originally, NSC transplantation was proposed as a means of replacing cells in central nervous system (CNS) diseases that result in cell loss (Einstein and Ben-Hur, 2008). Studies performed by Abbah et al., 2008 and Zhang et al., 2008 demonstrated the ability of transduced articular chondrocytes to survive and promote proteoglycan accumulation when transplanted into the intervertebral discs. These data support the potential of a cell-based gene therapy approach for disc repair.

One of the fields of medicine that has raised the most expectations in recent years is cell therapy with stem cells. The isolation of human embryo cells, the apparent and unexpected potentiality of adult stem cells and the development of gene therapy lead to imagine a hopeful future for a significant number of diseases that are at present incurable (Martin, 2007).

In the field of dermatology, tumor-associated antigens (TAA) are promising candidates as target molecules for immunotherapy and a wide variety of tumor-associated antigens have been discovered through the presence of serum antibodies in cancer patients. Conduction of dendritic cell therapy on malignant melanoma
patients proved shrinkage or disappearance of metastatic tumors (Yoshiura et al., 2005). Cell vaccination therapy in melanoma has now many years of experience. Initially, the treatment was based on the use of autologous or allogeneic inactivated tumor cells. This depends on the antigenicity of human tumor cells, which can be recognized by T lymphocytes and particularly by cytolytic T lymphocytes (CTL). This antigenicity of tumor cells lead to the development of therapeutic anti-cancer vaccines that induce tumor regressions or prevent the development of metastases in the vaccinated patients, with metastatic melanoma. Detailed immunological analyses with some of these vaccinated patients showed strong anti-tumor T cell responses and suggested that the main limiting factor for clinical efficacy is a phenomenon of resistance of the tumor to T lymphocyte attack (Baurain et al., 2008).

Scleroderma has an autoimmune-related pathogenesis, particularly in early illness. In this disease, stem cell therapy is a reasonable potential choice. Hematopoietic stem cell transplantation has being tested in prospective randomized controlled trials and appears to reset autoimmunity in systemic sclerosis (Ssc). Mesenchymal stem cells (MSC) may have an immunomodulatory role in autoimmune diseases such as scleroderma (Tyndall and Furst, 2007).

Recessive dystrophic epidermolysis bullosa (RDEB) is a severe inherited skin-blistering disorder caused by mutations in the COL7A1 gene that lead to reduced type-VII collagen and defective anchoring fibrils at the dermal-epidermal junction (DEJ). Presently there are no effective treatments for this disorder. Many studies have shown that intradermal injections of normal human fibroblasts can generate new human type-VII collagen and anchoring fibrils at the DEJ (Wong et al., 2008).

Most low grade lymphoma and chronic lymphocytic leukemia cells express monoclonal immunoglobulins (Igs) carrying either kappa (κ) or lambda (λ) light chains. T lymphocytes could be genetically modified to target the tumor-associated light chain, sparing B lymphocytes expressing the reciprocal light chain, and consequently reduce impairment of humoral immunity. T lymphocytes expressing the anti-kappa light chain showed
cytotoxic activity against Ig kappa positive tumor cell lines both in vitro and in vivo (*Vera et al.*, 2006).

Treatment of severe radiation burns remains a difficult challenge. Conventional surgical treatment (excision, skin grafting, skin or muscle flaps) often fails to prevent unpredictable and uncontrolled extension of the necrotic process. Some clinical cases in which surgery was combined with MSC therapy, proved good clinical outcome with no recurrence (*Bey et al.*, 2007).

There is a growing debate in the medical community over the efficacy and ethical implications of cell therapy. Much of the ethical debate revolves around the use of human fetal stem cells in treatment. While some cell therapy procedures have had proven success in clinical studies, others are still under continuous research to reach optimum benefits for all mankind (*Martin*, 2007).
Aim of The Work
Aim of the Work

The aim of this essay is to provide an updated review on cell therapy in dermatology & highlight the recent achievements in uses, applications and management of different dermatological diseases.
Review of Literature
Chapter I
Cell Therapy
REVIEW OF LITERATURE

CHAPTER 1

CELL THERAPY:

Cell therapy is the transplantation of human or animal cells to replace or repair damaged tissue and/or cells. Cell therapy is also referred to as "live cell therapy," "xenotransplant therapy," "cellular suspensions," "glandular therapy," or "fresh cell therapy". Cell therapy is, in effect, a type of organ transplant. The procedure involves the injection of either whole fetal xenogenic cells (e.g., from sheep, cows, pigs, and sharks) or cell extracts from human tissue. The latter is known as autologous cell therapy if the cells are extracted from and transplanted back into the same patient. Cell therapy technologies overlap with those of gene therapy, cancer vaccines, drug delivery, tissue engineering and regenerative medicine. (Martin, 2007).

HISTORY:

In the history of medicine, man has always tried to treat diseases with available materials starting from herbal plants, metals and metallic compounds and then antibiotics and anti-viral agents. Where these did not have a scope, he resorted to manual intervention in the form of surgery. Having expanded his knowledge in molecular medicine, he should resort to cell based therapy (Rajgopal, 2005).

The theory behind cell therapy has been in existence for several hundred years. The first recorded discussion can be traced to Phillippus Aureolus Paracelsus, a German-Swiss physician who wrote in his Great Surgery Book in 1536 that "the heart heals the heart, lung heals the lung, spleen heals the spleen; like cures like. In 1667, at a laboratory in the palace of Louis XIV, Jean-Baptiste Denis attempted to transfuse blood from a calf into a mentally ill patient, and since blood transfusion is, in effect, a form of cell therapy, this could be the first documented case of this procedure. In 1912 German physicians attempted to treat children with
hypothyroidism, with thyroid cells. In 1931, Dr. Paul Niehans, a Swiss physician, became known as "the father of cell therapy". After a surgical accident by a colleague, Niehans attempted to transplant a patient's severely damaged parathyroid glands with those of a steer. Niehans decided to dice the steer's parathyroid gland into fine pieces, mix the pieces in a saline solution, and inject them into the dying patient. Immediately, the patient began to improve and, in fact, lived for another thirty years (Martin, 2007).

The first published case of a patient receiving Hematopoietic stem cell transplantation (HSCT) as treatment for an autoimmune disease was published in October 1996 (Wall et al.) Since then, over 1000 patients have been transplanted for autoimmune diseases. In 2002, clinical trials were announced involving a French biotechnology company to develop cell therapy to treat heart disease. The cell therapy program would test the theory that cell therapy could reverse the damage done to heart muscle during a heart attack, stopping progression to congestive heart failure. Thus Cell therapy is starting to become a part of medical practice. (Menasche, 2002).

**PREPARATIONS:**

There are several processes to prepare cells for use. One form involves extracting cells from the patient and then culturing them in a laboratory setting until they multiply to the level needed for transplant back into the same patient. Another procedure uses freshly removed fetal animal tissue, which has been processed and suspended in a saline solution, either injected immediately into the patient, or preserved by being freeze-dried or deep-frozen in liquid nitrogen before being injected. Cells must be tested for pathogens before use. Methods of delivery of cell therapy range from injections to surgical implantation using special devices. (Martin, 2007).
**PRECAUTIONS and SIDE EFFECTS:**

Patients undergoing cell therapy treatments which use cells transplanted from animals or other humans run the risk of cell rejection. Some forms of cell therapy use special coatings on the cells designed to trick the immune system. There is also the chance of the cell solution transmitting bacterial or viral infections to the patient. Careful screening and testing of cells for pathogens can reduce this risk. The full range of possible side effects of the treatments are not yet known. These substances may have not been thoroughly tested to find out how they interact with medicines, foods, or dietary supplements. Anaphylactic shock, immune system reactions, and encephalitis are just a few of the known reported side effects in some patients to date. Women who are pregnant or breastfeeding should not use this method, as its possible effects on a fetus are unknown. The benign tumor glioneuronal tumor is a rare side effect of fetal stem cell therapy. Chondrocyte cell therapy used in knee joint repair may include tissue hypertrophy. Much of the ethical debate revolves around the use of human fetal stem cells in treatment, and the fact that these cells must be harvested from aborted fetuses but other sources of stem cells exist as the placenta, cord blood and fat removed by liposuction. Stem cells can also be genetically modified prior to transplantation. *(Witten, 2008).*
STEM CELL THERAPY

Stem cells are cells found in most, if not all, multi-cellular organisms. They are characterized by the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. Research in the stem cell field grew out after efforts by Canadian scientists McCulloch and James in the 1960s. The two broad types of mammalian stem cells are: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues.

Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies (Adewumi et al., 2007).

Stem cells are broadly categorized into Embryonic Stem Cells (ESCs) and Adult or Somatic Stem Cells (SSCs). ESCs can be derived in two ways: from embryo formed by the fusion of sperm and ovum, and through therapeutic cloning using Somatic Cell Nuclear Transfer (SCNT) technique (Rajgopal, 2005).

Embryonic stem cell lines are cultures of cells derived from the epiblast tissue of the inner cell mass of a blastocyst or earlier morula stage embryos. A blastocyst is an early stage embryo, approximately four to five days old in humans and consisting of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers:
ectoderm, endoderm and mesoderm. In other words, they can develop into all cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta. Nearly all research to date has taken place using mouse embryonic stem cells or human embryonic stem cells. Both have the essential stem cell characteristics, yet they require very different environments in order to maintain an undifferentiated state. Mouse ES cells are grown on a layer of gelatin and require the presence of Leukemia Inhibitory Factor (LIF). Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts and require the presence of basic Fibroblast Growth Factor (bFGF) (Takahashi and Yamanaka, 2006).

Adult stem cells are found in the mature, terminally differentiated tissues and are of two types: Hematopoietic Stem Cells (HSCs) and MSCs. HSCs are found in the bone marrow, peripheral blood, umbilical cord blood and fetal hematopoietic system. MSCs are found in the bone marrow as stromal cells, umbilical cord matrix, placental tissue, fetal tissue, fat and muscle. A subpopulation of MSCs known as Multipotent Adult Progenitor Cells are also identified in the bone marrow. Multipotent adult stem cells, also known as basal cells, have been known to exist in the basal layer of epidermis of skin and in the lining epithelium of gastrointestinal tract (GIT) and respiratory tract and in the epithelium of the limbus. More recently the discovery of stem cells in the post mitotic tissues, thought to be incapable of regeneration, like brain, myocardium and skeletal muscle has opened up exciting new possibilities (Witten, 2008).

Hematopoietic stem cell transplantation (HSCT) is a procedure in which progenitor cells capable of reconstituting normal bone marrow function are administered to a patient. This procedure is often performed as part of therapy to eliminate a bone marrow infiltrative process, such as leukemia, or to correct congenital immunodeficiency disorders (Jiang et al., 2002).
Both ESCs and adult stem cells are capable of indefinite self renewal; are capable of differentiation into specific functional cells and tissues and are capable of homing to areas of inflammation and injury. ESCs are pluripotent and are capable of differentiating into tissues derived from any of the germ layers (ectoderm, mesoderm or endoderm). MSCs also show pluripotency by differentiating into many tissues. This capacity of transdifferentiation makes them ideal for stem cell therapy. Viral transduction using adenoviruses in stem cells can generate stable clones and may be used in gene therapy. Multipotent adult progenitor cells may be turned into pluripotent stem cells similar to ESCs. Another remarkable aspect of stem cell therapy is the immune privilege enjoyed by the ESCs. Immune privilege is an experimentally defined phenomenon in which certain sites, cells, tissues and organs fail to obey the rules of transplantation immunology, including the possibility of rejection. Thus cells with such an ability are able to tolerate transplantation without eliciting an inflammatory immune response (Rajgopal, 2005).

The use of ESCs is however mired in the ethical controversy of destruction of the embryo while harvesting ESCs. Use of allogenic or autologous MSCs or HSCs will circumvent this ethical controversy easily. But they have to be expanded in numbers before use and their plasticity (differentiation potential) is less than that of ESCs. Apart from ethical problems, ESCs therapy also faces some biological problems. ESCs have a tendency to form teratomas and also exhibit chromosomal abnormalities. Maintenance and expansion of ESCs are presently done on feeder cell layer like mouse embryonic fibroblast layer. Such exposure to animal cell lines carries a risk of contamination with retroviruses and other pathogens (Martin, 2007).

Mesenchymal cell types could be isolated easily, and expansion in cell cultures is convenient, thus making them ideal candidates in autologous cell transplantation. Lorentz et al. In 2008 proved that human dermis fibroblastic mesenchymal cells possess stem cell like characteristics and are phenotypically similar to Adipose Derived Stem Cells (ADSC) Fig 1. Human adipose tissue was obtained from patients undergoing plastic surgery and human juvenile foreskin samples were obtained from patients undergoing circumcision. The surface antigen of both MSCs and ADSCs was
similar. They also proved that Fibroblast Mesenchymal Stem Cells (FMSC) are able to differentiate to adipogenic and osteogenic lineages. FMSC fulfil the three main characteristics of MSCs: They express homogenously all MSC related surface antigens, their cytoskeleton and matrix composition is similar to that of MSCs, they differentiate along the adipogenic and osteogenic cell lineages.

Adipose tissue has proven to serve as an abundant, accessible, and rich source of adult stem cells with multipotent properties suitable for tissue engineering and regenerative medical applications. ADSCs are most conveniently extracted from tissue removed during an elective cosmetic liposuction procedure but may also be obtained from resected adipose tissue. The number of stem cells that can be isolated per unit volume of lipoaspirate is approximately 10-fold greater than that from bone marrow. Lipoaspirate is processed into a heterogeneous, non-adipocyte cell population, referred to as the stromal vascular fraction (SVF). Aspirated adipose tissue is washed and digested with collagenase to yield a heterogeneous population from which ADSCs can be expanded. ADSCs have phenotypic and functional characteristics very similar to bone marrow–derived mesenchymal stem cells, and have an equal potential to differentiate into cells and tissues of mesodermal origin, such as adipocytes, chondrocytes, and osteoblasts. In 2007, Schaffler and Buchler proved that adipose-derived stem cells are able to differentiate into multiple cell lineages including cardiac myocytes. Hence, adipose-derived cells are emerging as a new source of adult stem cells for cardiovascular repair and many other medical applications.

Potential stem cell applications include cardiomyocytes for heart failure, dopaminergic neurons for Parkinson’s disease, neural cells for spinal cord injury, and islet cells for diabetes mellitus. Cardiomyocytes developed from stem cells contract spontaneously, express appropriate cell type markers, and express appropriate receptors for cardioactive drugs. Osteoblasts developed from stem cells have been shown to heal fractures in animal models. While not yet useful for liver disease, hepatocytes derived from stem cells facilitate drug discovery research by testing metabolism and
toxicity of new compounds. Neuronal ESCs develop synapses, produce a full spectrum of neurotransmitters, and exhibit normal electrophysiology. Engrafted dopaminergic cells have been shown to function and survive in patients with Parkinson's disease, but thus far have not improved patient outcomes. In a rat model of spinal cord injury, transplantation of oligodendrocytes into areas of acute contusion resulted in remyelination and partial functional recovery (Yu et al., 2007).
Chapter II
Dermatological Applications
CHAPTER 2

DERMATOLOGICAL APPLICATIONS:

1-MELANOMA:

Melanoma is a malignant tumor of melanocytes which are found predominantly in skin. Around 160,000 new cases of melanoma are diagnosed worldwide each year. It causes the majority of skin cancer related deaths. Malignant melanoma arises from melanocytes in a pigmented area as skin, mucous membranes, eyes, or CNS. Metastasis is correlated with depth of dermal invasion. With spread, prognosis is poor. A skin biopsy performed under local anesthesia is often required to assist in making or confirming the diagnosis and in defining the severity of the melanoma. The sole effective cure is surgical resection of the primary tumor before it achieves a Breslow thickness greater than 1 mm. Treatment beside surgery, is adjuvant treatment, chemo- and immunotherapy, or radiation therapy. Metastatic disease requires chemotherapy as dacarbazine (DTIC), immunotherapy with interleukin-2 or interferon, but is difficult to cure. The most common types of melanoma in the skin are: superficial spreading melanoma, nodular melanoma, acral lentiginous melanoma, lentigo maligna, lentigo maligna melanoma; invasive melanoma arising from a lentigo maligna and melanoma-in-situ (Autier, 2005).

In the 1940s and 1950s, the use of transplantable tumors proved that tumor transplants could be rejected in mice and that this rejection was mediated by the immune system, especially CTL, which express the molecular cluster of differentiation 8 (CD8). The antigenic peptide migrates to the surface of the cell where it can be recognized by the receptors of a specific CTL. Many tumors carry antigens that can serve as a target attack by CTL, immunization against them could lead to a tumour rejection in vivo. The purpose of these vaccinations is to allow the patients immune system to react against tumor antigens expressed by its own tumor, hoping to destroy tumor cells without affecting normal tissues (Baurain et al., 2008).
TAA are promising candidates as target molecules for immunotherapy and a wide variety of TAA have been discovered through the presence of serum antibodies in cancer patients. The TAAs of melanoma are: tumor specific mutated Ags (such as β-catenin), cancer –testis Ags such as Melanoma antigen family A (MAGE-1), HOM-MEL 40 and NY-ESO-1; and differentiation antigens such as tyrosinase. MAGE genes are not expressed in normal tissues. They are present in many tumors, including melanoma (Yoshiura et al., 2005).

In 2008, Baurain et al. underwent a clinical trial where 26 melanoma patients, known human leukocyte antigen (HLA) typing, were subjected to a tumor biopsy followed by reverse transcription polymerase chain reaction (RT-PCR) for expression of the gene that encodes MAGE. Then they received repeated immunizations, both intradermal and subcutaneous. Every patient underwent a tumor assessment and collection of blood lymphocytes before and after treatment. Significant tumor regression in seven patients was noticed (Fig2). Three of these seven responders had eliminated metastases completely. Regressions observed involved mostly skin and lymph node metastases. Pigmented spots could persist for months after the complete regression. The mechanism proposed was that a small number of CTL induced by the vaccine, migrates into the tumour and reactivates other anti-tumour CTL to destroy much of the tumour mass. Peptides associated with an immunological adjuvant, potentiates the response of T lymphocytes.

Cancer cells could avoid being destroyed by CTL. This can be due to the loss of expression of antigen by tumor cells, loss of expression of HLA molecules, the resistance of tumor cells to apoptosis, the production of factors interfering with the immune response, such as certain cytokines or constant presence of antigen inducing a phenomenon of depletion of CTL and malfunction or destruction of local tryptophan by cancer cells, as T cells are very sensitive to lack of tryptophan, which causes the end of their cell cycle. Vaccines have been generally well tolerated and reported side effects included local inflammatory reactions (Gimenez et al., 2009).
-Fig 1:

Human dermis fibroblastic mesenchymal cells possess ADSCs like characteristics.

- Fig 2:

Metastatic melanoma in 2 patients before and after treatment.
Another trial was on ten malignant melanoma patients treated by dendritic cell therapy, with shrinkage and disappearance of metastatic tumors with massive necrosis occurred in two patients. It was found that a 29-kDa protein, against which antibody was elicited by dendritic cell therapy was present in one of these two patients. Electrophoresis combined with Western blots revealed that the 29-kDa protein was carbonic anhydrase II (CA-II). Immunohistochemistry of the tumors and normal tissues showed that CA-II was expressed in the tumor vessel but not in normal vessel endothelium. Dendritic cells were prepared by culturing autologous monocytes with granulocyte-macrophage colony-stimulating factor (GMCSF) and interleukin 4 (IL4). Anti CA II antibody was positive in six of ten melanoma patients. CA II, thus was identified as an antigen which elicited humoral immune response in melanoma patients, recognized as TAA (Yoshiura et al., 2005).

CA-II has not generally been regarded as a tumor-associated protein because it is expressed in a wide variety of normal cells including erythrocytes, pancreatic epithelial cells, as well as those of the kidney and GIT. The presence of anti–CA-II antibody has been reported in some systemic autoimmune diseases, such as systemic lupus erythematosus, primary Sjögren's syndrome, progressive systemic sclerosis and dermatomyositis. Some types of cancer also have high CA II expression as brain tumors, leukemia, esophageal cancer, renal cell carcinoma, and lung cancer. CA inhibitors have been shown to inhibit growth and invasion of cancers including melanoma. Given that CA-II has a critical role in tumor angiogenesis, CA inhibitors may deteriorate tumor angiogenesis and cause tumor reduction or inhibition of tumor growth. The antibody status during therapy, may be useful as a marker of good clinical response to dendritic cell therapy (Yoshiura et al., 2005).

Hunder et al (2008) showed that the infusion of a clonal population of cluster of differentiation 4 (CD4)+ T cells with specificity for a single TAA caused complete regression of a tumor. During regression of the tumor, this clone appeared to have induced the patient's own T cells to respond to other antigens of his tumor. These findings support further clinical studies of antigen-specific CD4+ T cells in the treatment of malignant diseases. The patient was 52 years old when he presented with

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recurrent melanoma and pulmonary, left iliac and inguinal metastases. His tumor had not responded to high-dose interferon alfa, four cycles of high-dose IL-2, and local excision. Baseline staging with magnetic resonance imaging of the brain and computed tomography (CT) of the chest, abdomen, and pelvis showed metastatic disease in the posterior right pleura, right hilum, and left iliac region. There were no CNS metastases. CT scans obtained two months after the T-cell infusion revealed no evidence of disease, and follow-up after twenty two months showed no evidence of recurrence. Twenty six months after the T-cell infusion, the patient had received no other treatment and had normal function. There were no detectable acute or long-term autoimmune-related toxic effects (e.g., dermatitis, vitiligo, uveitis, and orchitis). Thus CD8+ cytotoxic T cells could be harvested from a patient with cancer, expanded in vitro, selected for specificity against a tumor-associated antigen, and infused back into the patient. Such autologous T cells have been shown in clinical trials to have a beneficial effect in some patients with cancer. The cytotoxic antitumor effect of CD8+ T cells depends on CD4+ T cells, which provide CD8+ T cells with growth factors such as IL-2 and can mediate the destruction of tumor cells either directly or indirectly. Growth factors such as IL-2 can act in an autocrine manner, which in principle would allow an infusion of CD4+ T cells to proliferate in the patient and stimulate endogenous antitumor CD8+ T cells.

2-SCLERODERMA:

SSc or scleroderma is a systemic connective tissue disease. This disease is found among all races worldwide, but women are four times more likely to contract scleroderma than men. Characteristics of SSc include essential vasomotor disturbances; fibrosis; subsequent atrophy of the skin, subcutaneous tissue, muscles, and internal organs (GIT, lungs, heart, kidney, CNS); and many immunologic disturbances accompany these findings. Typical scleroderma is classically defined as symmetrical skin thickening, with about 90% of cases also presenting with Raynaud's Phenomenon, nail-fold capillary changes, and anti-nuclear antibodies. There is no cure for scleroderma. Treatment focuses
on relieving symptoms and reducing the risk of complications. Localized skin changes may be treated with topical agents like moisturizers or corticosteroid medications. Ultraviolet light therapy has also shown some benefit. Vasodilators, may be prescribed to relieve Raynaud’s phenomenon. Avoiding exposure to cold and sunlight is also helpful. Angiotensin Converting Enzyme Inhibitors (ACEI) to control blood pressure and alleviate serious kidney complications. Nonsteroidal anti-inflammatory drugs (NSAIDs) for arthritis. Steroids, such as prednisone, may be used to decrease inflammation. D-penicillamine is thought to decrease the production of collagen and may delay the progression of the disease. Immunosuppressants like cyclophosphamide and methotrexate, may be needed for some patients. Thus treatment is tailored according to individual patient presentation (Bernatsky et al., 2009).

MSCs are multipotent stem cells that can differentiate into a variety of cell types. Cell types that MSCs have been shown to differentiate into in vitro or in vivo include osteoblasts, chondrocytes, myocytes, and adipocytes. MSCs have a large capacity for self-renewal while maintaining their multipotency. MSCs may have an immunomodulatory role in autoimmune diseases. They inhibit T-cell proliferation and formation of CTLs, induce a more anti-inflammatory environment in vitro through promoting formation of type 2 dendritic cells and regulatory T cells, as well as inhibition of type 1 dendritic cells, production of Interferon gamma (IFN-g), Tumor necrosis factor alpha (TNF-a) and IL-12. The therapeutic benefit is due to homing to inflamed tissue and the release of local cytokines and growth factors, resulting in local antiproliferative and immunomodulatory effects. They have immunosuppressive properties, for both T and B lymphocytes in mixed lymphocyte cultures (Ringdén et al., 2006).

Immunosuppressive agents such as cyclophosphamide have long been used to treat autoimmune diseases, but the dose is often limited by bone marrow suppression. Many years ago several groups considered adopting the oncological approach of myeloablative therapy followed by hematological rescue using either autologous or allogeneic hematopoietic stem cells to treat
severe, therapy-resistant autoimmune diseases. **Tyndall and Furst** in 2007 found, regarding autologous HSCT, that the first sixty five transplanted patients reported to the European League Against Rheumatism and European Group for Blood and Marrow Transplantation (EULAR/EBMT) database showed an improvement of 25% in the skin score in 70% of the patients, with a transplant related mortality (TRM) of 2.5%. Another thirty four poor prognosis SSc patients, underwent total body irradiation and cyclophosphamide, beside HSCT. After four years of follow up, an approximately 70% improvement in skin score and 55% improvement in function were noted. Routine use of ACEI during the transplant period was done. So the outcome of the study showed a significant number of SSc patients followed for ten years in which major regression of skin thickening has occurred as well as stabilization of lung function. In case of allogenic HSCT it is a more toxic treatment option, Graft-versus-host disease (GVHD) is a known complication.

Scleradactyly is a known complication of scleroderma, with poor circulation and infection it can progress to an ulcer and a resistant wound. In 2009, Roehr used autologous bone marrow-derived stem cells, mesenchymal stem cells that can differentiate into the full range of skin and muscle cell types. The cells were cultured ex vivo and their numbers were expanded greatly. A solution of the stem cells and fibrinogen was placed in one chamber of a double-chambered syringe, and the second chamber was filled with a solution of diluted thrombin. The two solutions combined when ejected from the syringe as a spray over the wound. The mix began to polymerize, and that clotting helped to hold the stem cells in place in the wound. The wound was then covered with two layer bioengineered skin, containing a layer of keratinocytes and a layer of fibroblasts. Cellular signaling molecules helped direct the stem cells to fill the gaps, restoring the entire tissue compartment. Pain relief was very rapid for patients receiving the procedure. With healing at four and eight weeks, the procedure was marked successful and raised many future hopes.

High-dose immunosuppressive therapy and autologous stem cell transplantation, commonly referred to as stem cell transplantation is an established treatment for a variety of haemato-oncological conditions. Recent studies have confirmed its potent clinical and immunological effects in rheumatic autoimmune diseases,
including severe diffuse SSc. With modifications of treatment protocols and more stringent selection of patients, the safety profile of stem cell transplantation has improved as expressed in lower treatment-related morbidity and mortality. Prospective, randomised trials are in progress in Europe and North America to compare the safety and efficacy of stem cell transplantation with conventional chemotherapy in patients with early diffuse SSc, on the premise that induction of remission in early disease can be achieved by stem cell transplantation as a means to interrupt fibrogenesis (Van Laar et al., 2008).

3- RDEB:

Epidermolysis bullosa (EB) is a group of autosomal dominant and recessive inherited bullous disorders characterized by the presence of extremely fragile skin and recurrent blister formation, resulting from minor mechanical friction or trauma. EB is classified into three major categories, including: EB simplex (intraepidermal skin separation), junctional EB (skin separation in lamina lucida or central basement membrane zone (BMZ), and dystrophic EB (sublamina densa BMZ separation). RDEB is a severe inherited skin blistering disorder caused by mutations in the COL7A1 gene, that lead to reduced type VII collagen and defective anchoring fibrils at the DEJ, characterized by widespread skin and mucous membrane fragility. Typically, blisters are followed by scarring and there is increased incidence of squamous cell carcinoma, which is the major cause of death in young adults with RDEB. Diagnosis is clinical, followed by skin biopsy and genetic testing.

Skin integrity and resistance to mechanical stress rely on the function of the DEJ zone, which anchors the epidermis to the underlying dermal matrix. The supramolecular cell adhesion complexes at the DEJ zone mediate interactions of the cytoskeleton in basal keratinocytes with the basement membrane and the extracellular anchoring fibrils, which emanate from the basement membrane into the dermis and entrap dermal collagen bundles, thus establishing stable dermal-epidermal cohesion. Presently there are no effective treatments for this disorder but cell-based therapy carries a great hope for DEB patients (Poocheron et al., 2008).
In 2008, by Poocheron et al. an important experiment was conducted. Intradermal injections of allogenic fibroblasts were given to RDEB patients. The result was increased type VII collagen at DEJ at two weeks and at three months, with increased anchoring fibrils. Injections led directly to better adhesion between the epidermis and underlying dermis Fig 3. Skin biopsies taken from sites injected with allogeneic fibroblasts showed less dermal–epidermal blistering. Electron microscopy (E/M) of the DEJ revealed a 1.5 fold increase in the number of anchoring fibrils. Fig 4a -4b. Allogenic fibroblast injections were not associated with any clinico-pathological inflammatory response. They exerted a paracrine effect on the keratinocytes to increase synthesis of mutant type VII collagen fragments, which if present in sufficient amount can reduce blistering.

In a collagen VII hypomorphic mouse, the phenotype closely resembled characteristics of severe human DEB. The mouse presented with skin fragility, nail dystrophy, digital malformations, pseudosyndactyly and growth retardation. Mouse received two intradermal injections of fibroblasts into a defined area on dorsal skin. The treatment resulted in both deposition of collagen VII at DEJ and functional restoration inspite of intense mechanical stress that failed to induce dermo-epidermal separation and blistering, all this persisted through the entire twenty one days observation period. Thus intradermal injections of fibroblasts resulted in neodeposition of collagen VII and functional restoration of the DEJ. Treatment of DEB with normal fibroblasts would appear to have major advantages compared with other forms of gene- or cell-based therapies. Fibroblasts are easy to obtain, cultivate, and store, and the delivery of these robust cells via intradermal injections is practical. Thus cell-based therapy for DEB showed that intradermal injection of fibroblasts into the hypomorphic mice not only augmented deposition of collagen VII at the DEJZ, but also appeared to improve dermal-epidermal cohesion. (Fritsch et al.,2008).
- Fig 3:

Allogeneic Cell Therapy for Epidermolysis Bullosa.

- Fig 4 a:

Transmission electron microscopy shows an increase in the number of rudimentary anchoring fibrils 3 months following intradermal injection of allogeneic fibroblasts. This figure illustrates the morphological appearances of the DEJ in skin biopsies performed in subject 4. (a) The ultrastructural appearances of normal skin show anchoring fibrils that insert into the lamina densa (arrows) and that have a fan-shaped appearance and central cross-
banding. (b) At baseline, skin of subject 4 demonstrates a few anchoring fibril-like structures extending from the DEJ (arrows). Many of these fibrils appear short, thin, and lack central cross-banding and fan-shaped appearance. (c) Three months following injection of unrelated allogeneic fibroblasts there is an increased number of anchoring fibrils along the DEJ in the skin of subject 4 (arrows), although most of these appear rudimentary and lack the morphologic characteristics of the normal skin anchoring fibrils.

-Fig 4 b:

Immunohistochemical labeling shows only transient inflammation following intradermal injection of allogeneic fibroblasts. This figure illustrates the immunohistologic findings in subject 5 using CD45 antibody (pan-T cell marker) and CD11c (dendritic cell marker). (a) CD45 staining of baseline skin reveals only a few lymphocytes in the dermis. (b) Two weeks following autologous fibroblast injection there is a slight increase in the number of lymphocytes in the papillary dermis. (c) Two weeks after injection of parental fibroblasts and also (d) unrelated allogeneic fibroblasts the number of dermal lymphocytes is increased.
Prompted by findings in animal studies that intradermal injections of normal human fibroblasts can generate new human type-VII collagen and anchoring fibrils at the dermal–epidermal junction, investigators injected cultured fibroblasts into small areas of skin in five patients with RDEB (two had severe Hallopeau-Siemens RDEB, one had moderate disease, and two had milder forms). Pre-injection biopsy samples showed marked reductions in anchoring fibrils in the three patients with moderate or severe disease and slight reductions in the two patients with milder disease. Each patient then received intradermal injections of cells from three sources — their own cultured fibroblasts, fibroblasts from one parent, and fibroblasts from an unrelated donor. At two weeks, unrelated donor and parent cell injection sites demonstrated increased type-VII collagen in all patients; the increase persisted at three months in the three patients with mild or moderate disease. The patients’ own fibroblasts had no effect on type-VII collagen production. The number of anchoring fibrils increased after injection but had a rudimentary appearance. Fibroblasts had disappeared from all sites by two weeks, but the persistent increase in collagen at three months suggests that the patients’ cells had been stimulated to produce increased amounts of abnormal type-VII collagen. Subjects with milder disease seemed to respond better than did those with more-severe RDEB. Clinically, skin fragility appeared to improve at the sites of parental or unrelated donor fibroblast injection (Wong et al., 2008).

4-PSORIASIS:

Psoriasis is an inflammatory disease that manifests most commonly as well-circumscribed, erythematous papules and plaques covered with silvery scales. It is a form of hyperproliferation of epidermal keratinocytes combined with inflammation of the epidermis and dermis. Family history is common, suggesting a genetic component in many cases. Common triggers include trauma, infection, and certain drugs, as B blockers, chloroquine, lithium, ACEI and indomethacin. Symptoms are usually minimal with occasional mild itching, but cosmetic implications may be major. Arthritis develops in 5 to 30%
of patients and can be disabling. Diagnosis is based on appearance and distribution of lesions. Treatment modalities include emollients, vitamin D analogues, retinoids, tar, anthralin, corticosteroids, phototherapy, and when severe, methotrexate, retinoids, biologics, or immunosuppressants (Gelfand et al., 2005).

Biologics are designed to treat psoriasis and psoriatic arthritis by targeting overactive cells in the body. Some biologics target one type of immune cells (T cells), while others target the chemical messengers released by activated T cells. The biologic medications have been investigated by the National Institute for Health and Clinical Evidence (NICE) who have issued guidelines on when they can be prescribed. Current approved biologic agents for psoriasis include agents that inhibit TNF alpha (eg, etanercept, infliximab, adalimumab) or T-cell activation (eg, alefacept, efalizumab). (Fernández-Cruz et al., 2008).

The pathogenesis of psoriasis has been reported to be driven by activated T cells or antigen presenting cells, which release a number of cytokines and chemokines. These then bind to surface receptors and signal keratinocytes to hyperproliferate and abnormally differentiate. TNFα is a major mediator in the pathogenesis of psoriasis. However there is also the speculation that increased reactive oxygen species (ROS) production and deficient function of cellular antioxidant system play a critical role in the etiology of the disease (Grove, 2007).

In 2008, the role of ROS and TNFα in psoriasis was investigated by Chen et al. TNFα treatment induced H2O2/ROS generation and increased levels of IL 8 and IL 6, while catalase prophylaxis significantly reduced the amount of H2O2 and related ROS, presented in TNFα treated keratinocytes. Thus it was proposed that targeted antioxidant prophylaxis, with a transducible catalase derivative is worth considering as a potential treatment modality.

Angiogenesis is a complex process that is regulated by pro- and anti-angiogenic factors. These factors can emanate from diverse sources including cancer cells, stromal cells, blood and extracellular matrix. Their relative contribution is likely to change with tumor type and tumor site. Vascular endothelial growth factor
(VEGF) is now well confirmed as the primary and the most potent inducer of angiogenesis. To activate cellular signaling pathways, VEGF binds to receptor kinases VEGF-R1, R2 and R3. It then promotes several events required for the formation of new blood vessels, such as endothelial cell survival, proliferation, migration and vascular permeability. Activation of endothelial cells, leads to the secretion of enzymes which degrade the extracellular matrix (ECM) and hence promote metastasis. Similarly it promotes survival by inducing Bcl-2 ,which is the prototype for a family of mammalian genes and the proteins they produce. They govern mitochondrial outer membrane permeabilization. Besides being a potent mitogen for macrovascular cells derived from arteries, veins and lymphatics, it is also highly involved in a number of angiogenic related disorders including inflammatory diseases, rheumatoid arthritis, psoriasis, retinopathies and age related macular degeneration. Neovascularization and increased vessel permeability are being recognized as major causes of VEGF related pathogenesis. Therefore, inhibition of VEGF pathway is a strategy being widely pursued to provide new therapeutics for the treatment of VEGF related disorders. Over twenty compounds with anti-angiogenic properties ranging from VEGF neutralizing antibody, soluble receptors, receptor antagonists or tyrosine kinase inhibitors (TKIs) are either approved or are currently under clinical studies (Pourgholami and Morris, 2008).

5- SCLEROMYXEDEMA:

Scleromyxedema is a rare chronic fibromucinous disorder associated with a monoclonal gammopathy, that can have devastating clinical manifestations including, sclerosis of the skin with progressive pharyngeal and upper airway involvement, resulting in high mortality due to respiratory complications. It is also known as papular mucinosis , characterised by deposits of mucin in the skin. Most cases are characterized by dermal fibroblast proliferation and mucin deposition, associated with plasma cell dyscrasia. Two forms are present : localised and generalised forms. The localised form has a more favourable course compared to the generalised form, which can involve other organs and is sometimes fatal. Conventional treatment has been unsatisfactory (Mulekar,2003).
Many therapeutic approaches have been tried. These approaches include treatment with retinoids, orthovoltage radiation, electron beams, high-dose dexamethasone, psoralen UV-A (PUVA), plasmapheresis, extracorporeal photophoresis, dermabrasion, and carbon dioxide laser excision. More recently, improvements have been reported with the use of thalidomide. Topical tacrolimus reportedly has been successful in treating localized disease in few patients. Increasing evidence supports intravenous Ig as an effective and relatively safe treatment for both cutaneous and extracutaneous manifestations of scleromyxedema, including the dermato-neuro syndrome (Rey and Luria, 2009).

In an experiment by Lacy et al; 2005, six patients with scleromyxedema were referred for treatment with high-dose chemotherapy, in the form of conditioning regimen with Melphalan, and autologous peripheral blood stem cell transplant (PBSCT). A complete response was defined as a lack of detectable monoclonal protein in serum and urine samples by immuno-electrophoresis and immuno-fixation, as well as normalization of the bone marrow plasma cells, all stable for at least four weeks. Hematologic responses were seen in four patients, including two complete responses and two partial responses. Improvement of skin involvement was clear as skin nodules and nodular plaques disappeared Fig5. Improvement in extra-cutaneous manifestations were also manifest. Thus autologous PBSCT should be considered for patients with scleromyxedema.

In another trial by Donato et al., 2006, Seven of eight patients were treated with high-dose melphalan (180 mg/m² intravenously) and autologous PBSCT, with marked improvement of GIT, CNS, pulmonary manifestations. Five patients obtained a cutaneous complete remission and two patients had partial remissions. Three patients with slight progression in the skin at twelve, eight, and four months after treatment, received a second cycle of high-dose melphalan and had further symptomatic improvement. Thus high-dose melphalan followed by autologous transplantation appeared effective for improving the symptoms and systemic manifestations of scleromyxedema.
Fig 5:

Scleromyxedema:

A, C, and E, pretransplantation; B, D, and F, posttransplantation.
6-VITILIGO:

Vitiligo is a disorder consisting of areas of macular depigmentation, commonly on extensor aspects of extremities, on the face or neck, and in skin folds. Age of onset is often in young adulthood and the condition tends to progress gradually with lesions enlarging and extending until a quiescent state is reached. Etiology is unclear. It is both familial and acquired. Proposed mechanisms include autoimmune destruction of melanocytes, reduced survival of melanocytes, and primary melanocyte defects. Occasionally, vitiligo occurs after a direct physical injury to the skin (e.g., as a response to sunburn). Patients may associate the onset of vitiligo with emotional stress. Some patients have antibodies to melanin. Up to 30% have other autoimmune antibodies or clinical autoimmune endocrinopathies as Addison's disease, diabetes mellitus, pernicious anemia, and thyroid dysfunction, leading to speculation that vitiligo is an autoimmune disease. The strongest association is with hyperthyroidism, Graves' disease and hypothyroidism, Hashimoto's thyroiditis. First-line treatment is topical corticosteroids. Calcineurin inhibitors as tacrolimus and pimecrolimus, and psoralens plus ultraviolet A are commonly used. For severe widespread pigment loss, depigmentation of residual patches of normal skin may be done with hydroquinone (Millington and Levell, 2007).

The history and techniques of grafting of cultured melanocytes were described in 2007 by Czajkowski as follows, the in vitro expansion of epidermal cells was introduced by Rheinwald and Green in 1975 for the first time and was initially used for the treatment of burn patients. Now the culturing of human cells is one of the most common techniques used worldwide. The expansion of melanocytes by culturing enables treatment of larger areas. The time needed for cell culturing is about four weeks. The method of harvesting and culturing of autologous melanocytes was published by Lerner and colleagues in 1987. They injected the cultured melanocytes into the achromatic skin lesions, where the melanocytes have produced melanin like pigment in healthy skin. The result of this procedure was repigmentation of depigmented skin with a very similar colour.
like the colour of normally pigmented skin. This technique was successfully reproduced and used also by Olsson and Juhlin from Sweden in 1993. The modification of this technique with the use of E-YAG laser for recipient skin ablation was published by Kaufmann from Frankfurt in 1998. The perforated absorbable hyaluronic acid matrix (Laserskin®) was used as a cell carrier. The successful transplantation of autologous melanocytes into the skin denuded by CO₂ laser was published by team of Prof Chen from Taiwan in 2004. Interesting technique for cultured melanocytes implantation was used by a team of doctors from Faculty Hospital in Brno, Czech republic, melanocytes were injected into skin by dermojet. Probably the first commercial product for surgical treatment of vitiligo by cultured melanocytes is MelanoSeed (Bio Tissue Technologies, Freiburg, Germany). The melanocytes are obtained from the full thickness skin biopsy, melanocytes are cultured for twenty eight days and the final product, suspension of cultured melanocytes in fibrin, is applied on skin denuded by dermabrasion.

Transplantation of autologous melanocytes is an additional option in patients with stable vitiligo that no longer respond to conventional therapy. A total of thirty three paired, symmetrically distributed leukodermic lesions, all resistant to therapy, were observed in twenty eight patients. Nineteen patients appeared to have a stable vitiligo, whereas there was doubt about the stability of the disease in nine patients. After laser ablation, a hyaluronic acid–enriched cellular graft, that increased the viscosity of the cellular graft was applied to one lesion while the paired lesion received placebo. The donor site was a shave biopsy specimen of approximately 2 cm² taken from the patients' normally pigmented gluteal region under local anesthesia. Three weeks later all lesions were exposed to Ultra Violet (UV) irradiation twice per week for approximately two months. In stable type, repigmentation of at least 70% of the treated area was achieved. Thus transplantation resulted in repigmenting most of the treated area. Repigmentation was primarily caused by the transplanted melanocytes Fig6 (Van Geel et al., 2004).

Similar clinical trial by Mulekar in 2003 included one hundred eighty four patients treated with melanocyte-rich cell transplantation technique over a period of three years. The
patients were divided into three groups: segmental, focal and generalized. All were stable type vitiligo, they were observed up to one year postoperatively. The cell suspension was applied to the derm–abraded depigmented skin area with collagen dressing applied. In the generalized vitiligo group sixty five patients showed excellent pigmentation. In segmental group thirty six patients also showed excellent pigmentation. In the long run this method should prove to be a cosmetically acceptable, fast and cost effective therapy.

7-GVHD:

GVHD is a disease of modern medicine. It most commonly occurs in bone marrow transplant patients receiving an allogeneic transplant of immunocompetent lymphocytes. The donor CTL attack the recipient’s organs including skin, GIT, liver, and mucous membranes. It may occur in 50% of recipients of allogenic bone marrow transplants. GVHD may also occur after materno-fetal blood transfusions, intrauterine exchange transfusions, and administration of non-irradiated blood products to patients with metastatic malignancies or with immunodeficiencies. Classically, acute and chronic disease has been based on time combined with clinical and histological parameters. Primary therapy for chronic GVHD is administration of cyclosporine and prednisone. Agents such as mycophenolate mofetil, sirolimus, and rituximab have also led to response rates of more than 60% in patients with steroid-refractory chronic GVHD (Reddy et al., 2008).

The discovery that MSCs can strongly inhibit T cell proliferation in vitro and in vivo and exert similar inhibitory effects on B cells, dendritic cells, and natural killer cells has highlighted the potential for clinical translation of these cells as a new class of stem cell therapy for autoimmune disease, organ transplantation and treatment of GVHD. Even though the mechanism underlying these immunosuppressive effects of MSCs has not been clearly defined, their immunosuppressive properties are already being exploited in the clinical setting. Most of these early clinical studies are investigating the effect of MSCs in suppressing GVHD after allogeneic HSCT (Zhang et al., 2009).
MSC is a very promising treatment for severe steroid-resistant acute GVHD as MSC have immunomodulatory effects. A study by Ringdén et al. in 2006 showed the effect of MSC infusion on GVHD. MSC was given to eight patients with steroid-refractory grades III-IV GVHD and one who had extensive chronic GVHD. Acute GVHD disappeared completely in six of the eight patients.

5-WOUND HEALING:

Wound healing is the body's natural process of regenerating dermal and epidermal tissue. A set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage. These events overlap in time and may be artificially categorized into separate steps: the inflammatory, proliferative, and remodeling phases. In the inflammatory phase, bacteria and debris are phagocytized and removed, and many factors are released that cause the migration and division of cells involved in the proliferative phase. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis (Santoro and Gaudino, 2005).

Efficient wound healing involves numerous factors, especially sufficient supply of growth factors and adequate circulation of oxygenated blood (Suh et al., 2005). Also the therapeutic management of severe radiation burns remains a challenging issue. In 2007, Lataillade et al. reported an innovative therapeutic strategy applied to the victim of a radiation accident. The approach combined numerical dosimetry guided surgery with cellular therapy using MSCs, capable of proliferating extensively and giving rise to skeletal tissue. The aim of MSCs was to deliver to the lesion site trophic factors able to favor the healing of impaired tissue. Autologous bone marrow total cells were obtained from iliac crest aspirations Fig 8.
-Fig 6:

Test site (arrow) (left wrist) before cellular grafting.

Test site (arrow) (left wrist) 3 months after cellular grafting.
Fig 7:

(a) Generalized vitiligo of 8 years duration in a 28-year-old male.
(b) Left ankle 1 year after transplantation.

(a) Segmental vitiligo of 13 years duration in a 29-year-old female.
(b) One year after transplantation.
(a) Generalized vitiligo of 10 years duration in 24-year-old female.
(b) Left ankle one year after transplantation.

-Fig 8:

Bone marrow stem cell harvesting.
Local administration of MSCs by injection was done in circles around the lesion at the cutaneous and muscular levels and in the wound bed. The clinical evolution, regarding pain and healing was favorable and no recurrence of radiation inflammatory waves was observed during the eleven month follow up period.

In 2005, Suh et al created a full thickness excisional wound on the back of three mice. Immediately after surgery, Endothelial Progenitor Cells (EPC), Human Dermal Microvascular Endothelial Cells (HDMEC) or Phosphate Buffer Saline (PBS) were injected into the three different created wounds. EPC transplantation accelerated the rate of wound closure as early as day 3 after surgery Fig 9. EPC injected wound showed a markedly increased number of monocytes/macrophages, that increased the supply of cytokines and growth factors in the wounded tissue. Also there were numerous newly formed capillaries in the EPC treated group. EPC assisted wound healing is potentially a therapeutic approach for the treatment of chronic wounds, which remain a major clinical problem, especially in diabetic patients.

Apligraf® Fig 10 a is a unique, advanced biological skin repair therapy, and is created from biological ingredients found in healthy human skin. It has an epidermal layer formed from human keratinocytes and a dermal layer composed of human fibroblasts in a bovine type I collagen matrix. A study compared the efficacy and safety of Apligraf in combination with standard therapy versus standard therapy alone in the treatment of neuropathic diabetic foot ulcers. Efficacy was assessed by time to complete wound healing (by twelve weeks) and incidence of complete wound closure (at twelve weeks). It was an international multi-center, randomized, controlled study. The median time to healing was eighty four days in the Apligraf group, thus by twelve weeks, Apligraf subjects had achieved complete wound closure compared with standard therapy subjects. The study suggested that the use of Apligraf resulted in a higher incidence of wound closure and is best used to heal ulcers such as diabetic foot and venous leg ulcers Fig 10b (Edmonds, 2009).
Representative images show wound healing in mice treated with EPCs, HDMECs, or PBS. Wounds were photographed at the times indicated, from days 0–12.

-**Fig 10 a:**

Apligraf for diabetic ulcers.
- Fig 10 b:

- Invitro apligraf.

- Preclinical grafting efficacy(invivo).
Biobrane and Integra were the first Food and Drug Administration (FDA) approved biologically based wound dressings. Biobrane is a temporary dressing for a variety of wounds including ulcers, lacerations, and full-thickness burns. It may also be used on wounds that develop on donor sites. Biobrane uses an ultra-thin silicone film and nylon fabric, which is partially imbedded into the film. The nylon material contains a gelatin derived from pig tissue that interacts with clotting factors in the wound. Integra Dermal Regeneration Template was approved in 1996 to treat full-thickness and some partial-thickness burns. Integra is a two-layer membrane. The bottom layer, made of shark cartilage and collagen from cow tendons, acts as a matrix onto which a person's own cells migrate over two to three weeks. The cells gradually absorb the cartilage and collagen to create a new dermis. This bottom layer is a permanent cover. The top layer is a protective silicone sheet that is peeled off after several weeks. A very thin layer of the person's own skin is then grafted onto the neo-dermis. Both Biobrane and Integra use animal tissue; other approved cellular wound dressings are made with human tissue. One of these products is OrCel. Approved by the FDA in 2001 to treat donor sites in burn patients, OrCel is made of living human skin cells grown on a cow collagen matrix (Cetinkale et al., 2006).

6-SCARRING ALOPECIA:

Scarring alopecia, also known as cicatricial alopecia, refers to a collection of hair loss disorders that may be diagnosed in up to 3% of hair loss patients. It occurs worldwide in otherwise healthy men and women of all ages. Each specific diagnosis within this category is fairly rare, but some examples include dissecting cellulitis, eosinophilic pustular folliculitis, follicular degeneration syndrome, folliculitis decalvans, lichen planopilaris, and pseudopelade of Brocq. Scarring alopecia may also be part of a much larger condition such as chronic lupus erythematosus. Scarring alopecias are classified into primary and secondary types according to the initial site of inflammation. In primary scarring alopecias, the hair follicle is the main target of destruction; the term secondary cicatricial alopecia implies that follicular destruction is not the primary pathologic event. While there are many forms of scarring
alopecia, the common theme is a potentially permanent and irreversible destruction of hair follicles and their replacement with scar tissue (Moure et al., 2008).

Primary cicatricial alopecias (PCAs) are a poorly understood group of disorders that result in permanent hair loss. Clinically, they are characterized not only by permanent loss of hair shafts but also of visible follicular ostia along with other visible changes in skin surface morphology, while their histopathological hallmark usually, although not always is the replacement of follicular structures with scar-like fibrous tissue. As hair follicle neogenesis in adult human scalp skin is not yet a readily available treatment option for patients with cicatricial alopecias, the aim of treatment, currently, remains to reduce symptoms and to slow or stop PCA progression, namely the scarring process. Early treatment is the key to minimizing the extent of permanent alopecia (Harries et al., 2008).

Cotsarelis in 2007 found that wound healing in a mouse model triggered an embryonic state in the skin that lead to sending stem cells to the area of injury by introducing Wnt signaling proteins to the wound. The Wnts are a network of proteins implicated in hair-follicle development. It was found that researchers could take advantage of the embryonic genes to promote hair-follicle growth, thus making skin regenerate instead of just repair. Conversely by blocking Wnt proteins, production of hair follicles was stopped in healed skin, while increasing Wnt signaling doubled the number of new hair follicles. This can be used to manipulate hair follicle regeneration leading to novel ways to treat hair loss and hair overgrowth and find a possible treatment for male pattern baldness, and other hair and scalp disorders including scarring alopecia (Fig. 11).

Follicle cells were cultured and implanted to mouse ears and footpads in an experiment by McElwee et al. in 2003. Dermal papiller (DP)-derived cells and cells from the peribulbar dermal sheath (DS) induced new hair follicles in both implanted ears and footpads, while nonbulbar dermal sheath cells did not. DS cells were characterized in vivo and in vitro by low alkaline phosphatase activity in contrast to high alkaline phosphatase in DP cells. The results indicated that transplanted DP and DS cells were equally capable of DP formation and hair follicle induction. This suggested
that the DP and peribulbar DS may be functionally similar. Alkaline phosphatase expression may be utilized as a simple marker to identify hair follicle mesenchyme derived cells with hair follicle inductive abilities.
-Fig 11:

Scientists succeed in hair follicle regeneration in an animal model.
Chapter III
Aesthetic Applications
The key components of facial aging are soft-tissue atrophy, gravitational descent, and loss of skin tone. An accurate assessment of the relationship of these factors will determine the role of soft-tissue augmentation through the use of fillers. The majority of facial volume loss through aging is attributable to fat loss. Rejuvenation is defined as skin remodeling that gives the patient a more youthful look by diminishing the signs of aging and the damage caused by years. Continued patient demands had fueled the introduction of a wide variety of injectable fillers, which include dermal and subdermal fillers with varying degrees of viscosity and duration of benefit. Fillers include collagen, silicone, hyaluronic acid, Polymethylmethacrylate (PMMA), microscopic calcium hydroxylappatite particles and recently natural materials as autologous fat and autologous Platelet Rich Plasma (PRP) injections (Sapijaszko, 2007).

Introduction of cultured fibroblasts, both autologous and allogenic increases the dermal fibroblast population and this can correspond to an overall increase in the collagen content of the dermis as well as remodeling of preexisting collagen. One revolutionary product Intercytex (ICX-RHY) is a suspension of cultured allogenic neonatal human fibroblasts. Although allogenic do not illicit an immune reaction even after multiple applications. Biopsies showed thickening of both the epidermis compared to placebo and a marked increase in the number of dermal fibroblasts present Fig.12 (Lowe, 2008).
Fig 12:

Soft Tissue Fillers

24-year-old with an early naso-labial fold and some soft tissue loss lateral to the chin.

Seen at six months with excellent response to Intercytex.
The appropriate timing for cell isolation in autologous cell therapy, is a major concern. Many of the putative target diseases arise with old age and previous evidence, mainly from animal models, suggests that the stem/progenitor cell pool decreases steadily with age. Studies with human cells have been generally hampered to date by poor sample availability. In recent years, several laboratories have reported on the existence, both rodents and humans, of skin-derived precursor (SKP) cells with the capacity to generate neural and mesodermal progenies. This easily obtainable multipotent cell population has raised expectations for their potential use in cell therapy of neurodegeneration. However, the clear understanding of the spatiotemporal abundance and phenotype of human SKPs is still lacking (Xie et al., 2008).

In 2009, Gago et al. showed an analysis of human SKP abundance and in vitro differentiation potential, by using SKPs isolated from four distinct anatomic sites (abdomen, breast, foreskin, and scalp) from one hundred and two healthy subjects aged eight months to eighty five years. Human SKP abundance and differentiation potential decrease sharply with age, being extremely difficult to isolate, expand, and differentiate when obtained from the elderly. It was suggested that preserving human SKP cell banks early in life would be desirable for use in clinical protocols in the aging population.

ADSCs and their secretory factors show promise for application in cosmetic dermatology, especially in the treatment of skin aging. ADSCs and their secretory factors can stimulate collagen synthesis and migration of fibroblasts during the wound healing process (Zhu et al., 2009). Park et al. in 2008 analyzed secretory factors of ADSCs and intradermally injected ADSCs and conditioned media on the back of a micropig. In addition, intradermal injections of purified autologous processed lipoaspirate cells were tried with the photoaged skin of one patient. The patient had two successive injections at two weeks interval and showed improvement in texture and wrinkles two months after the second injection. These results were demonstrated through a comparison of medical photographs and dermal thickness using a high frequency ultrasonograph Fig 13. This proved that ADSCs produce many useful growth factors, increase collagen production in animal study and reverse skin aging in human trial. Some ADSCs growth
factors were VEGF, Basic Fibroblast Growth Factor (BFGF), Transforming Growth Factor (TGF).

In an in-vitro study, adipose tissue samples were obtained through elective liposuction with informed consent, from healthy candidates. After isolation of the ADSCs, they were depicted by flow cytometric characterization. For the analysis of the secretory factors, ADSCs were plated on a 100-mm dish overnight with a control medium and were cultured in a Dulbecco’s modified Eagle’s medium. They were then collected after 72 hours of culture, centrifuged for 5 minutes, and filtered through a 0.22-mm syringe filter. The concentration of cytokines and extracellular matrix proteins were measured using sandwich enzyme-linked immunosorbent assay kits (Park et al., 2008).

The process of autologous cell regeneration (ACR) is a facet of cell therapy and regenerative medicine. PRP can be defined as an autologous concentration of human platelets in a small volume of plasma. PRP gel consists of fibrillar element and cellular component that contains human platelet cells Fig 14 a. It is initiated when concentrated, multiplied and expanded fibroblasts from the same person is injected into a designated dermal recipient region as the face Fig 14 b to enhance neocollagenosis and skin tightening. The recipients resident cells are activated by biologically active growth factors, derived from the activated platelets in the PRP through a paracrine effect. These growth factors influence activation of macrophages and stem cells in the recipient site, thus, tissue regeneration is facilitated and wound healing is provided. Being autologous, problems of immunological rejection and disease transmission were overcome (Du Toit et al., 2007 a).

PRP possesses unique growth factors that stimulate fibroblasts, keratinocytes and myoblasts ex-vivo in tissue culture, allowing three dimensional cell proliferation within the fibrin gel. Some factors recognized are Platelet Derived Growth Factor (PDGF), TGF and Epidermal Growth Factor (EGF). To facilitate the biological process of tissue regeneration and remodeling, PRP can be used alone or in combination with cells, such as keratinocytes, fibroblasts, myoblasts or ADSCs. The platelet fibrin
gel serves as a cell vehicle carrier. Collagen production is enhanced in vitro by PRP. If the platelets in the blood clot were activated by either calcium chloride or thrombin, release of growth factors occurs. These factors can increase the rate of collagen deposition, angiogenesis, fibroblast proliferation and extracellular matrix synthesis. Thus strong evidence was provided for the application of PRP in facial rejuvenation in chronological and photo-aged face with wrinkles and skin sagging (Du Toit et al., 2007 b).
Clinical study using intradermal injections of autologous PLA cells. Medical photographs of periorbital wrinkle were taken (A, before treatment; B, after treatment) and dermal thickness was measured by an ultrasonograph (C and D). Improved skin texture and dermal thickness (2.054mm vs. 2.317mm) were clearly observed 2 months after injection of PLA cells (B and D).
-Fig 14:

Platelet Rich Plasma Technique:

a-Activated platelets.

b-Face injection.
No problems of immunological rejection and transmission of diseases were noted but, regarding facial rejuvenation, initial results were short lived and inconsistent. Drawbacks if used in facial area included the potential to microthrombosis in the region of the anterior facial vein, closed compartment syndrome, release of pro-inflammatory proteolytic activators from leucocytes and hemosiderin deposition that lead to hyperpigmentation. Cosmetic physicians can combine PRP treatment with adjunct radiotherapy, Intense pulsed light (IPL) or laser therapy for long lasting results (Du Toit et al., 2007 a).

Facial lipoatrophy is a disfiguring and socially disabling problem. There is no medical treatment to correct facial lipoatrophy. In 2008, Yoshimura et al., found that autologous lipoinjection is a promising treatment for soft tissue augmentation and they conducted a study in Cell Assisted Lipotransfer (CAL), where ADSC poor fat was converted to ADSC rich fat by supplementing with cells freshly isolated from the adipose tissue. Three patients underwent conventional lipoinjection (non CAL), while three patients underwent CAL. One of the six patients had Parry Romberg syndrome, the other five had lupus erythematosus profundus. In the CAL strategy, autologous ADSC were used to enhance angiogenesis, improve the survival rate of grafts, and reduce post operative atrophy. Photographs were taken before and after treatment. All patients showed cosmetic improvements, but CAL had a better clinical improvement score than non CAL. Also for cosmetic breast augmentation Yoshimura et al., suggested that CAL is safe and may be superior to conventional lipoinjection.
Chapter IV
Non-Dermatological Applications
CHAPTER 4

NON- DERMATOLOGICAL APPLICATIONS:

1-NEUROLOGY:

NSCs are defined by their ability to self renew and differentiate into cells of glial and neuronal lineages and to populate developing or diseased CNS regions. In 2008, Einstein and Ben-Hur proved that undifferentiated murine NSCs when transplanted into the newborn cerebellar cortex, don’t replace host Purkinje Neurones (PNs), but contact imperiled PNs and support their mitochondrial function, leading to the rescue of host PNs. They also rectify excessive tissue Plasminogen Activator (tPA) to normal levels but in the untreated cerebellum of chosen mice there was severe depletion of PNs. Thus the NSCs treated mice retained near normal motor coordination, and showed normal mitochondria.

2-ORTHOPEDICS:

Back pain associated with symptomatic disc degeneration is a common clinical condition. Articular chondrocytes transplantation has been used widely to repair articular cartilage defects in sports medicine. Autologous articular chondrocytes eliminate the risk of tissue rejection or disease transmission (Tallheden et al., 2006).

In 2008, Zhang et al. demonstrated the ability of transduced articular chondrocytes to survive and promote proteoglycan accumulation when transplanted into the intervertebral disc. They supported the potential of a cell based gene therapy approach for disc repair. In their study the effects of placing articular chondrocytes transduced with one of two growth factors: bone morphogenetic protein 10 (BMP10) and bone morphogenetic protein 7 (BMP7), compared with chondrocytes carrying a marker gene: green fluorescent protein (GFP) as a control, were evaluated. The expression of BMP7 in the disc led to a significant increase in the proteoglycan content of the nucleus pulposus (NP). This cell
based gene delivery approach may prove useful in the future as a strategy for disc repair.

Another orthopaedic problem is frozen shoulder, an intimacy presenting with chronic fibrosis of the shoulder joint capsule. Persistent anatomic defects remain after rotator cuff repair. It was postulated that biologic augmentation of the rotator cuff repair with PRP may improve healing. The activated PRP in gel form is gently delivered and evenly distributed onto the repair site, where it adheres to the raw surface. Growth factors will be delivered and promote healing. The process would usher new dimensions in shoulder surgery (Du Toit et al., 2007b).

3-ONCOLOGY:

Dendritic cells (DC) are recognized as the most potent antigen-presenting cells with the ability to stimulate naive resting T cells and to initiate primary immune responses. Many trials indicate that immunotherapies utilizing DC presenting tumor-associated antigens can safely be administered to patients with cancer and induce significant immunologic and clinical responses. DC based immunotherapy is a promising approach to augment tumor antigen-specific T cell responses in prostate cancer patients (Kaskel et al., 2007). Waeckerle et al. in 2006 determined the immunostimulatory capacity of autologous DC pulsed with multiple T cell epitopes derived from four different prostate-specific antigens in patients with advanced hormone-refractory prostate cancer. The vaccination elicited significant cytotoxic T cell responses against all prostate-specific antigens tested.

Despite the use of hormonal therapy and chemotherapy, metastatic breast cancer is rarely cured. Sixteen patients with metastatic breast cancer that had progressed after conventional treatment, received allogeneic lymphocytes, as adoptive cellular therapy after a reduced-intensity chemotherapy conditioning regimen and allogeneic HSCT from human leukocyte antigen–matched siblings. Objective tumor regressions occurred after twenty eight days observation period in six patients and were
attributed to allogeneic lymphocyte infusions. Two of these responding patients had disease progression post–allogeneic HSCT before subsequent tumor regression occurred. Thus allogeneic lymphocytes can induce regression of advanced metastatic breast cancer (Bishop et al., 2004).

Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV)–related malignancy expressing EBV antigens that are possible targets of cell therapy, including latent membrane protein 2 (LMP2). A clinical trial of EBV-targeted cell therapy with autologous virus-specific CTLs for NPC refractory to conventional treatments was conducted. Cell therapy with EBV-targeted autologous CTLs was proved to be safe, induced LMP-2-specific immunologic responses, and was associated with objective responses and control of disease progression in patients with stage IV NPC resistant to conventional treatments Fig15 (Comoli et al., 2005).

Renal cell carcinoma (RCC) has been shown to be susceptible to immunotherapeutic treatment strategies. In a recent study, patient-derived tumor cells were fused with allogeneic DC to elicit anti-tumor activity against RCC. DC from healthy donors were fused with primary RCC cells from ten patients. Clinically, ten patients were vaccinated with allogeneic DC/RCC fusion vaccine. In vivo, no serious adverse effects, toxicity, or signs of autoimmune disease were observed after vaccination therapy. One of ten patients exhibited a partial response with regression of lung metastases. Six patients showed stable disease with stabilization of previously progressive disease after eighteen months follow up period (Zhou et al., 2009).
Fig 15:

Nasopharyngeal cancer:

Objective response to Epstein-Barr virus (EBV)–targeted cytotoxic T-lymphocyte (CTL) therapy in patient 7. (A and B) Computed tomography–aided positron emission tomography imaging before T-cell therapy shows massive liver involvement. (C and D) After 3 months of EBV-targeted CTL therapy, reduction of size and isotope uptake of NPC lesions was detected.
Sickle cell disease is one of the world's most common inherited anemias, resulting in chronic hemolytic anemia and intermittent vaso-occlusion. The only cure for sickle cell disease is bone marrow HSCs transplantation, which can legitimately be called adult stem cell therapy. The main issues restricting the use of stem cells from an HLA-matched donor are immunologic and could be overcome if a patient's own somatic cells could be reprogrammed to make HSCs. Humanized mouse model of sickle cell anemia, can be rescued after transplantation of hematopoietic progenitors derived in vitro from autologous induced pluripotent stem cells. The red-cell abnormalities, hemolytic anemia, and associated pathologic features were corrected. The main problem was that the reprogrammed stem cell like cells used in the experiment were not the equivalent of the naturally occurring long term repopulating stem cells (Higgs, 2008).

Most low grade lymphoma and chronic lymphocytic leukemia (CLL) cells express monoclonal Igs, carrying either κ or λ light chains. Since the malignant cells in a given individual will express either κ or λ light chains, redirected T lymphocytes were isolated from CLL patients and programmed to kill primary κ+ B CLL cells, but they did not kill λ+ B CLL cells after incubation (Vera et al., 2006). Thus adoptive transfer of T lymphocytes genetically modified is an attractive approach for the treatment of B cell derived malignancies. Vera et al. demonstrated this both in vitro and in a xenograft model. Transgenic T cells were able to significantly control growth of κ+ tumor cells for more than three weeks after one single dose of T cells.
Summary
Cell therapy describes the process of introducing new cells into a tissue in order to treat a disease. It is considered a type of organ transplant. The theory behind cell therapy has been in existence for several hundred years. Paracelsus is considered the father of this branch in medicine. The procedure involves the injection of either whole animal cells or cell extracts from human tissue, which is either autologous or allogenic cell therapy.

Many processes prepare cells for use. One form involves extracting cells and then culturing them in a laboratory setting to multiply and then transplant them back into the same patient. Another procedure uses freshly removed fetal animal tissue. Many hazards are encountered including cell rejection, allergic reactions, hazards of infections and of course the ethical debate about using fetal cells after abortion.

Many applications of cell therapy has proven efficacy. Dermatological applications include its use in treating different diseases. Melanoma is a worldwide fatal type of cancer. Many tumors carry antigens that can serve as a target attack by CTL, immunization against them could lead to tumor rejection, and that is the basis of vaccination against melanoma. Dendritic cell
therapy is a proven way of treating melanoma, even after metastasis. Infusion of T cells with specificity for a single tumor-associated antigen caused complete regression of the tumor. TAA are promising candidates as target molecules for immunotherapy, as well as the infusion of a clonal population of CD4+ T cells with specificity for a single TAA caused complete regression of the tumor. Scleroderma patients were subject for cell therapy using MSCs that have immunomodulatory role, especially if combined with immunosuppressive agents.

In EB injection of allogenic fibroblasts improved the dermal-epidermal cohesion. Psoriasis and scleromyxedema are two difficult diseases were scientists tried cell therapy with PBSCT and improvement of disease manifestations resulted. Transplantation of autologous melanocytes is an additional option in patients with stable vitiligo that no longer respond to conventional therapy. In the long run this method should prove to be a cosmetically acceptable, fast and cost effective therapy.

MSC is a very promising treatment for severe steroid-resistant acute GVHD as MSC have immunomodulatory effects. Other important uses of cell therapy is its role in wound healing, as EPC transplantation increased the supply of cytokines and growth factors in the wounded tissue, resulting in chronic wound healing, especially in diabetics.
Introduction of cultured fibroblasts, both autologous and allogenic increased the collagen content of the dermis as well as remodeled preexisting collagen. ADSCs and their secretory factors can stimulate collagen synthesis. Thus both fibroblasts and ADSCs show promising results. But the use of PRP was an innovative, simple and cheap technique to restore what the skin loses by aging. PRP has unique growth factors that stimulate fibroblasts, keratinocytes and myoblasts allowing cell proliferation.

Non dermatological uses of cell therapy cover many aspects of modern medicine. Starting from PNs activation, chondrocytes transplantation in disc repair to its role in cancer management, including renal cell carcinoma, breast and prostate cancer that changed the life of many victims worldwide.
Conclusion
CONCLUSION

- Cell therapy is a unique mechanism of management of dermatological and non dermatological diseases affecting mankind.
- Cell therapy is simple, effective and safe method of medical intervention.
- Cell therapy is in its way to acquire confidence among scientists and physicians worldwide.
- Cell therapy is starting with humble steps in Egypt, it is recommended to expand its use in different fields and on many patients.
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Arabic Summary
العلاج الخلوي هو زرع خلايا بشرية أو حيوانية لاستبدال أو أصلاح الأنسجة التالفة. هناك عدة طرق لإعداد خلايا لاستخدامها مثل استخراج خلايا من المريض وبعد ذلك تحفظ في أحد المختبرات حتى تتضاعف إلى المستوى المطلوب للزرع ثم إعادتها إلى المريض. اجراء آخر يستخدم خلايا من الجنين في الأنسجة الحيوانية، والتي تم تجهيزها وعلقت في محلول ملحي. استخدمت هذه التقنية بنجاح لإعادة بناء الغضاريف التالفة في المفاصل، وصلاح اصابات النخاع الشوكي، وتقوية ضعف جهاز المناعه، وعلاج امراض المناعه الذاتية مثل مرض الايدز، ومساعدة المرضى الذين يعانون من اضطرابات عصبية مثل مرض الزهايمر، ومرض باركنسون، ومرض السرطان.

استخدامات أخرى أظهرت نتائج إيجابية في علاج مجموعة كبيرة من الأمراض المزمنه مثل تصلب الهراناين، والعيوب الخلقية ، وبعض انواع السرطانات المميتة كسرطان الثدي والبروستاتا. واحد من مجالات الطب والتي أثارت أكثر التوقعات في السنوات الأخيرة هو العلاج الخلوي مع الخلايا الجذعية. عزل خلايا الجنين البشري ووضع العلاج الجيني يؤدي نما إلى تصور مستقبل مفعم بالأمل بالنسبة لعدد كبير من الأمراض التي لا شفاء منه في الوقت الحاضر.

والهدف من هذه المقالة هو تقديم استعراض مستوفي على العلاج الخلوي في علم الأمراض الجلدي للتنظيف الضوء على المنجزات التي حققها هذا العلاج مؤخرًا في الاستخدامات والتطبيقات والشفاء من مختلف الأمراض الجلدية.
من الأمراض المهمة والتي حيرت العالم كلها ورم الخلايا الصبغية، الميلانوما وفيها تم استخدام العلاج بالخلايا المتشرحة الزوائد على شكل تطعيم في جرعات متباينة وقد اثبتت التجارب النجاح. وهناك العديد من الأمراض الجلدية التي تم الاحذاف فيها بالعلاف الخلوى كمرض تصلب الجلد و الصدفية والتجارب تفيد بوجود تعميم هذا العلاج على عينات أكبر من المرضى.

اما عن مرضا البهاق فقد بدا العمل في هذا المجال منذ اثقلتين عاما و هذه الفترة كانت كافية ليحصل زرع الخلايا الصبغية على ثقة الأطباء في عدد من بلاد العالم. واحد مات في هذا المجال هو اخذ الخلايا من الشخص نفسه ويزرع ذاتي.

التنام الجرواح المزمنة كجروح مرضى السكر تستجيب جيدا للعلاج بزرع خلايا جذعية وخلايا قرنية تعيد تشكيك نسيج الكولاجين والشعيرات الدموية.

اما مجال التجميل واعادة نضارة الوجه المتضرر بفعل الزمن والشمس فإنه نصيب كبير حيث يتم حقن خلايا البلازما بعد تدويرها وتشبيطها تفرز مواد منشطة للجلد وللانسحة الضامة فتكون النتيجة مساوية لجراحات التجميل التقليدية لكن بدون مخاطر الجراحة أو التعرض للتخدير الكلى.

ونحن هنا ننصح باجراء المزيد من البحوث لضمان تعميم هذا النوع من العلاج الآمن والرخيص الذي يسمح لعدد كبير من مرضى الأمراض الجلدية وعدد من الأمراض الأخرى التي احتار الأطباء في علاجها بالطرق التقليدية.
العلاج الخلوي في الأمراض الجلدية

مقالة مقدمة للحصول على درجة الماجستير
في الأمراض الجلدية والتناسلية

مقدمة من
الطبيبة دعاء حسين جودة
بكالريوس الطب والجراحة

تحت إشراف:
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