INTRODUCTION

The inevitable process of aging is a complicated process comprising intrinsic (genetic) and extrinsic (environmental) factors ***(Oikarinen 1990; et al., 2001)***. Intrinsic, also known as chronological aging is a term used to describe structural, functional and metabolic changes in the skin, paralleling the degenerative and age-related changes in other body systems ***(Uitto 1986; Guinot et al., 2002)*.** The process of intrinsic aging normally starts in the mid-20s but the signs are typically not visible for decades. It is determined by the genetic background. Genetics, of course, influence our resistance to extrinsic factors ***(Gilchrest 1992; Nagashima et al., 1999)***.

Intrinsic aging occurs universally in individuals of all racial and ethnic groups with all skin types. Intrinsically aged skin is characterized by generalized fine wrinkling, dryness, thinning, hypopigmentation and seborrhoeic keratosis. Additionally, there is loss of the underlying fat resulting in loss of firmness on the hands and neck ***(Gilchrest and Rogers, 1993)***. A number of extrinsic factors often act together with the normal aging process to prematurely age our skin. Most premature aging is caused by sun exposure. Other external factors that prematurely age our skin are repetitive facial expressions, gravity, sleeping positions, smoking and outdoors lifestyle ***(Koh et al., 2002)***.

Photodamage affects all races and skin types. Signs of photoaging may begin at an early age, as evidenced by freckles following ultraviolet light exposure. The clinical manifestations of photodamage may differ in lighter skin compared to darker skin types ***(Grimes et al., 2008)***.

Based on a person's complexion and responses to sun exposure, the different skin types are classified according to Thomas B. Fitzpatrick ***(Fitzpatrick, 1998)*** comprising 6 different skin types:

- Type I: Very fair/pale white skin that always burns and never tans.
- Type II: Fair skin that always/easily burns and poorly/sometimes tans.
- Type III: Medium/darker white skin that initially/sometimes burns and always tans.
- Type IV: Olive/light brown skin that minimally burns and always tans.
- Type V: Brown skin that rarely burns and always tans.
- Type VI: Dark brown or black skin that never burns and always tans.

In individuals with Fitzpatrick's skin types I to III or lighter complexioned races the clinical signs of photoaging

include wrinkles, laxity, dyschromia and textural alterations tending to appear as early as the second and third decade of life. It also may be accompanied by premalignant and malignant skin lesions including actinic keratosis, basal cell carcinoma, squamous cell carcinoma and melanoma ***(Grimes, 2004)***.

Darker racial ethnic groups have a higher content of epidermal melanin. In addition, fibroblasts are large and active often giving rise to a thicker dermis. The photoprotective effects of melanin in darker racial ethnic groups retard many of the early signs of aging. Hence photoaging changes are minimized in deeply pigmented skin. It manifests in the form of mottled pigmentation, texturally rough skin and fine wrinkles ***(Kaidbey et al., 1979)***.

Glogau classified the severity of photodamage based on the extent of epidermal and dermal changes. Severity of photodamage is categorized from I through IV, ranging from mild to moderate to advanced to severely photodamaged skin ***(Glogau, 1994)***.

Stem cells are undifferentiated cells, which have the important properties of self-renewal and differentiation. Mesenchymal stem cells or MSCs are partly differentiated, multipotent adult (tissue) stem cells that can be retrieved from the bone marrow and the stromal fraction of adipose tissue. They can differentiate into a variety of cells including

osteocytes, chondrocytes, adipocytes, hepatocytes, endothelial cells and epithelial-like cells ***(Zhang and Fu, 2008)***.

Forty years after the identification of bone marrow stem cells (BMSCs), a new era of active stem cell therapy has opened, with the discovery of adipose derived stem cells (ASCs). The abundance of stem cells harvested from adipose tissue has relative advantages in accessibility and abundance compared to other kinds of stem cells. Adipose-derived stem cells are easily isolated from the stromal vasculature of subcutaneous adipose tissue by liposuction with a minimally invasive procedure. Percentage of MSCs vary in different tissues. They demonstrate versatility in multilineage differentiation, implicating great clinical potential for MSCs in replacement/regeneration of diseased tissues/cells ***(Cheng et al., 2014)***.

MSCs set up a regenerative micro-environment which is anti-apoptotic, anti-scarring, angiogenic and mitotic for tissue intrinsic progenitors. These immune and trophic activities result from the secretion of powerful bioactive molecules that, in combination, support the local regenerative effect. The MSCs reside in every tissue of the body and function as perivascular cells (pericytes) until a focal injury occurs. At sites of injury, the pericytes are released and function as MSCs that provide molecular assistance in activities leading to tissue regeneration. Such assistance involves many tasks involving immune-protection and trophic activities provided by the MSCs.

Although it is proposed that all MSCs are pericytes and have common functional capacities, it is expected that MSCs from different tissue locations or anatomical sites of injury will not be identical. Thus, adipose-derived and bone marrow-derived MSCs naturally reside as pericytes and have different functional capacities ***(Da Silva Meirelles et al., 2008)***.

Regeneration therapy using adipose derived stem cells has received attention in the treatment of various dermatologic diseases. It was initially thought that the main potential of ASCs for regenerative medicine approaches was intimately related to their differentiation capability. Although this is true, there has been an increasing body of literature describing the trophic effects of ASCs on the protection, survival and differentiation of a variety of endogenous cells/tissues. Moreover, they have also shown to possess an immunomodulatory character. This effect is closely related to the ASCs' secretome and the soluble factors found within it. Molecules such as hepatocyte growth factor (HGF), granulocyte and macrophage colony stimulating factors, interleukins (ILs) 6, 7, 8 and 11, tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), adipokines and others have been identified within the ASCs' secretome ***(Kim et al., 2011)*.**

In vivo studies have shown that MSCs can transdifferentiate into tissue-specific cells in response to cues

provided by different organs ***(Jiang et al., 2002a)***. It was shown that MSCs may come to express keratin 14, a keratinocyte marker, when cultured in vitro. In wounds, MSCs have shown the capacity to differentiate into multiple skin cell types including endothelial cells, pericytes, and monocytes/ macrophages ***(Anjos-Afonso et al., 2004; Silva et al., 2005)***.

Therefore, MSCs can be considered non-hemopoietic multipotent stem cells that are capable of differentiating into both mesenchymal and non-mesenchymal lineages ***(Giordano et al., 2007)*.**

AIM OF THE WORK

The aim of this thesis is:

- Clinical and histological evaluation of the therapeutic potential of BMSCs and ASCs in the treatment of aged skin.
- Comparative assessment of their efficacy.

Chapter 1
SKIN AGING AND PHOTODAMAGE

Aging is a progressive process involving reduction in maximal function and reserve capacity of the whole organ. It is a consequence of both genetics and cumulative environmental effects ***(Yaar et al., 2002)***.

Human skin, like all other organs, undergoes chronological aging or intrinsic aging. In addition, unlike other organs, skin is in direct contact with the environment, and therefore undergoes aging as a consequence of environmental damage i.e. extrinsic aging ***(Fisher et al., 2002)***.

Intrinsic Aging:

Cutaneous aging is a complex biological phenomenon consisting of two components: intrinsic aging and extrinsic aging. Intrinsic aging is manifested primarily by physiologic alterations; causing structural and functional changes in all layers of the skin and is largely genetically determined ***(Balin and Pratt, 1989)***.

Despite the fact that it usually begins in the mid 20s and is a continuous process, the signs of intrinsic aging are not usually visible for decades ***(Sjerobabski–Masnec and Situm, 2010)***.

- **Etiology and Pathophysiology:**

The process of aging in the skin is similar to the one taking place in most internal organs and is thought to involve the following theories:

- *Cellular Senescence*

Cellular aging is the process by which a cell can no longer replicate; known as replicative senescence. A hallmark of replicative senescence is the shortening of telomeres, the specialized structures found at the ends of eukaryotic chromosomes that help ensure chromosomal stability ***(Buckingham and Klingelhutz, 2011)*.**

Telomerase is a special reverse transcriptase that stabilizes telomere length by adding to the ends of chromosomes. It is important in ensuring the complete replication of chromosome ends by preventing them from shortening during cell division ***(Blackburn et al., 1997)***. It is important to note that the epidermis is one of the few regenerative tissues to express telomerase ***(Boucamp, 2005)***.

Human aging can be triggered by two main mechanisms: telomere shortening and DNA damage. These two mechanisms act cooperatively to increase the overall level of genomic instability, triggering the onset of human aging phenotypes ***(Ding and Shen, 2008)***.

- *Alterations of Energy Metabolism in Cutaneous Aging*

Human skin tissue is exposed to abundant endogenous and environmental stress factors, therefore it is highly dependent on energy supply in order to combat cellular deregulation and/or to repair damage. Cellular energy levels decline during intrinsic aging and extrinsic aging as well, and consequently the capacity of the skin to counteract environmental stress declines with aging ***(Birch-Machin, 2006)***.

As an organ ages, either by extrinsic or intrinsic aging, there is a significant decline in mitochondrial function and cellular energy balance ***(Papa, 1996; Zwerschke et al., 2003)***. More than 100 mitochondrial DNA (mtDNA) mutations have been found in patients with mitochondrial disease, and some of them also occur in aging human tissues ***(Wallace, 1992)***. The incidence and abundance of mutant mtDNAs are increased with age, and are much more than nuclear DNA mutations ***(Linnane et al., 1989; Richter, 1995)***.

The increase in reactive oxygen species (ROS) production is a likely promotor for additional mtDNA damage and accumulation of mtDNA mutations, as a result of oxidative damage to DNA ***(Lu, 1999)***.

- *Reactive Oxygen Species:*

Free reactive oxygen species are generated in the skin by several different processes, with exogenous stress following UV-irradiation being the most dominant generator of ROS in UV-exposed skin ***(Ames et al., 1993)***.

High ROS concentrations, resulting from either increased production or decreased detoxification, can cause oxidative damage to various cellular components, ultimately leading to cell death ***(Droge, 2002)***. Beyond damage to mitochondria, age-related features often associated with excess ROS are accumulation of oxidized intracellular proteins with age, the decrease of fluidity of cellular membranes with age, and malfunctioning of the connective tissue remodeling process due to increased activity of extracellular matrix-degrading metalloproteinases ***(Huber et al., 1991; Stadtman, 1992; Nelson and Melendez, 2004)***.

- *Glucose-driven Intrinsic Aging:*

Glucose is a vital cellular fuel. However, based on the accelerated rate of aging seen in diabetics, chronic glucose exposure has long been known to affect how the body ages by a process called glycation ***(Van Boekel and Hoenders, 1992)***. Strong scientific evidence indicates that glycation reactions are promoted by oxidative stress, and in turn they lead to the further production of reactive oxygen species in the skin ***(Kikuchi et al., 2003; Crisan et al.; 2013)***.

- *Hormonal Influence:*

With aging, there is a decline in the level of sex hormones (estrogen, testosterone, dehydro-epiandrosterone sulfate), growth hormone as well as other hormones such as melatonin, insulin, cortisol and thyroxine ***(Phillips et al., 2001; Chahal and Drake, 2007; Makrantonaki et al., 2010)***.

Balance is critical in the realm of hormones, and these particular hormones have great influence on the skin. With declining estrogen levels, skin cellular renewal becomes sluggish, resulting in thinning of the epidermal and dermal layers. Capillary blood circulation velocity decreases significantly and the ability of the skin to maintain hydration, strength and elasticity suffers as a result ***(Raine-Fenning et al., 2003; Verdier-Sevrain, 2007)***.

Extrinsic Aging:

Extrinsic aging is accelerated aging that is superimposed on intrinsic effects of age that result from several exogenous insults to the skin ***(Sjerobabski-Masnec and Situm, 2010)***. Examples of which are: excessive use of alcohol, severe physical and psychological stress, overeating versus caloric restriction, gravitational forces, environmental pollutants, solar radiation including infrared and ultraviolet radiation, and environmental oxidants such as cigarrete smoke, ozone (O_3) and oxides of nitrogen ***(Wang et al., 1993; Uitto, 1997;***

Berneburg et al., 2000; Kelly et al., 2003). Of these external factors, sun exposure is considered to be, by far, the most significantly injurious insult to the skin. Indeed, 80% of facial aging is believed to be due to chronic sun exposure ***(Baumann, 2007)***.

- **Etiology and Pathophysiology:**

  - *Exposure to Solar radiation (Photoaging)*

On areas of the body that are less covered by clothing (i.e., face, nape of the neck, hands and forearms), the harmful effects of the sun and natural aging superimpose, leading to more pronounced alterations in the appearance of the skin indicating that photoaging includes both photodamage and chronological aging ***(Laure Rittie et al., 2010)***.

✶ **Infrared Radiation and Skin Aging:**

Schieke *et al.* reported in 2002 that low, physiologically relevant doses of infrared A (IRA: 760 – 1,440 nm) lead to a disturbance of the dermal extracellular matrix. IRA irradiation results in an induction of Matrixmetalloproteinase-1 (MMP-1) in vitro in human dermal fibroblasts, while expression of the respective tissue inhibitor TIMP-1 was not increased. This finding has, since then, been confirmed in independent studies by different workgroups in vitro and in vivo ***(Kim et al., 2006; Schroeder et al., 2008)***.

Matrixmetalloproteinases (MMPs) are calcium-dependant zinc-containing endopeptidases, involved in normal and pathological events in almost all human tissues ***(Nair et al., 2012; Chaussain et al., 2013)***. They are responsible for the degradation of extracellular matrix components such as collagen and elastin. MMPs are expressed primarily by fibroblasts but also by macrophages and keratinocytes and their expression is modulated by cytokines. Under physiological conditions, MMPs are part of a coordinate network and are precisely regulated by their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs). The unbalanced activity of MMPs with excessive proteolysis is thought to be a major pathophysiological factor in extrinsic skin aging ***(Fisher et al., 1997; Brenneisen et al., 2002)***. It is evident that the activity of the MMPs is one of the keys of the skin aging and that these enzymes have thus to be the target of therapeutic activities ***(Philips et al., 2011)***.

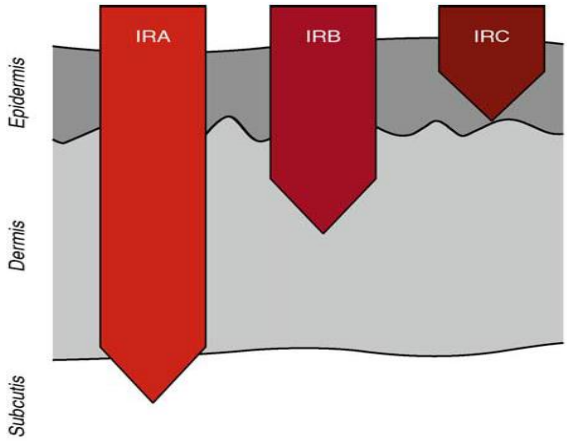

Figure (1): Skin penetration of infrared radiation. Different wavelengths of natural and artificial radiations have different penetration capabilities. IRA penetrates well into the skin, approximately 50% of IRA is absorbed in the dermis. IRB reaches the dermis as well, while IRC is nearly completely absorbed in the epidermis ***(Shroeder and Krutmann, 2010)***

✶ Ultraviolet Radiation and Skin Aging

Ultraviolet radiation (UVR) is the most significant environmental insult to the skin. The stratospheric ozone blocks the radiation whose wavelength is below 290 nm, effectively preventing the entire UVC spectrum and part of the UVB spectrum from reaching human skin ***(Berneburg et al., 2000)***.

The long-wave UVA radiation (320–400 nm) enters the deep dermis, while UVB light (280–320 nm) is absorbed mainly in the epidermis, especially in the keratinocytes and melanocytes. The direct interaction of UVB with cellular DNA induces damage of DNA strands. UVA radiation also damages the DNA but less than UVB radiation ***(Griffiths et al., 1998)*.**

UVB and, to a lesser degree, UVA induce various skin pathological conditions, including erythema, edema, hyperplasia, ‘‘sunburn cell’’ or UV-induced apoptotic cell formation, photoaging, and photocarcinogenesis ***(Kelly et al., 2003)***. The cumulative effects from repeated exposures to suberythemal doses of UVB, UVA, and IRA in human skin are involved in the process of photodamage ***(Schroeder et al., 2008)***.