An Analysis of the Rejuvenative Effect of Mesenchymal Stem Cells on Aged Skin: Comparative Evaluation of Two Types; Bone Marrow-Derived and Adipose-Derived Stem Cells

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Background/Aim:
Aging is a biological process that induces changes to the structural integrity and physiological function of skin. The aim of this study was to investigate the potential therapeutic effect of stem cells in reversing skin aging and to determine which type would be more efficient in achieving better results.

Abbreviations:
ASCs: Adipose Derived Stem Cells; BMSCs: Bone Marrow Derived Stem Cells; FBS: Fetal Bovine Serum.

Keywords:
Aging, photo damage, adipose derived stem cells [ASCs], bone marrow derived stem cells [BMSCs], morphometric analysis, collagen, melanin

Subjects and Methods:
Autologous ASCs and BMSCs were obtained from 10 participants and were re-injected into sites with visible signs of aging. Clinical evaluation was done pre-treatment as well as post-treatment and punch biopsies were taken from the dorsi of the hands for histological and morphometric analysis.

Results:
Both types of stem cells achieved similar clinical results. Morphometric analyses of the specimens were also similar for both types regard the collagen area percent and elastic fiber area percent. The detected increase in the ASCs group was higher than its BMSCs counterpart but comparison of the values was found to be statistically non-significant. In addition, ASCs group showed regeneration of skin appendages and an increase in epidermal thickness and epidermal melanin which was not observed in the BMSCs group.

Conclusion:
This study sheds the light on the anti-aging effects of stem cells particularly ASCs that prove to have a favorable skin regenerative and protective quality over BMSCs.

Introduction:
The inevitable process of aging is a complicated process comprising intrinsic [genetic] and extrinsic [environmental] factors[1,2]. Intrinsic aging occurs universally in individuals of all racial and ethnic groups with all skin types[3]. A number of extrinsic factors often act together with the normal aging process to prematurely age our skin[4]. 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 lifestyle [5]. Stem cell-based therapy is becoming a promising new approach in almost every medical specialty. Over the past few years there has been tremendous scientific activity focused on this area of research [basic, preclinical as well as clinical], and rapidly growing
evidence is accumulating to support the therapeutic potential of stem cells in tissue engineering and cutaneous wound healing.[6-11]. The results of those studies reveal that stem cells are capable of promoting wound healing and skin regeneration and re-epithelialization through their differentiation into multiple skin cell types and also, activation of dermal fibroblasts. This study was conducted to investigate the reparative effect of stem cells on aged skin and compare the therapeutic potential of ASCs and BMSCs in alleviating skin damage brought upon by age particularly photoaging.

Subjects and Methods:

Ten female participants were selected from the Dermatology Outpatient Clinic of Ain Shams University Hospital during the period from April 2012 to March 2014. All were healthy non-smokers showing clinical signs of photo aging. Ages eligible for this study range from 40 to 50 years of age. Subjects suffering from any chronic debilitating condition or any systemic disease, subjects on blood thinning medications and oral contraceptive pills, subjects receiving topical or systemic treatment for skin aging, and pregnant and lactating females were excluded from our study. The study was approved by the ethical committee of Ain Shams University and fulfilled all the ethical aspects required in human research. All the studied women were given a detailed description of the procedure to be performed and each gave an informed written consent.

Procedures and Treatment Protocol:

All the subjects underwent general anesthesia in order to obtain the two types of mesenchymal stem cells needed for our comparative study. BMSCs were obtained via a bone marrow aspirate from the posterior iliac crest using an aspiration needle. It was then processed using Harvest SmartPreP™ 2 Centrifuge System to concentrate the mononuclear cell constituent of the bone marrow. The bone marrow aspirate concentrate system produces the highest cell yields available while concentrating the full complement of cells and keeping them in their natural plasma. The bone marrow aspirate concentrate was mixed with the subject’s growth-factor rich plasma and then injected subcutaneously in the dorsum of the left hand of all the subjects and the left side of the face in the area showing pigmentary changes in five of the subjects. The adipose tissue required was obtained by tumescent liposuction. It was then injected as it is; unprocessed; in the dorsum of the right hand of all the subjects and the right side of the face subcutaneously in the area showing pigmentary changes in five of the subjects. All the aforementioned procedures were carried out in a single sterile operating room setting.
Skin Biopsies:

Two punch biopsies were obtained from each subject from the dorsi of both hands using a 3 mm punch biopsy probe. Another specimen was taken two months after the stem cell injection treatment. The specimens were prepared for histopathological evaluation, immunohistochemical staining and morphometric analysis using an image analyzer.

Subjective Assessment:

Clinical evaluation was performed using Griffiths’ photonumeric scale at baseline and two months post injection treatment based on: wrinkling on the dorsum of the hand, solar lentigines and mottled hyperpigmentation for the hands. Parameters of photodamage accounted for in the face were melasma, solar lentigines and mottled hyperpigmentation. Each parameter of photodamage present was given a score from 0-9: 0 = none, 1-3 = mild, 4-6 = moderate, 7-9 = severe. Subjects were asked to grade their satisfaction with the outcome on a scale from 1 to 5 with 1 denoting no satisfaction and 5 denoting marked satisfaction.

Histological Results

The H&E stained slides were examined microscopically and the special-stained slides were subjected to morphometric analysis for the objective evaluation of their collagen content as well as their elastic fibers and melanin content.

Microscopic examination of specimens belonging to Group III [ASCs-treated group] showed cut sections of sweat glands and their ducts as opposed to the absence of such signs in the BMSCs-treated group. In addition, an increase in epidermal thickness was detected in comparison to specimens belonging to Group I before the injection treatment, which was also absent in the BMSCs-treated group.
Hands were similar before the treatment. By comparing the pre-
according to Griffiths’ photonumeric scale, on both right and left
III [20%]. From a clinical standpoint, the scores for photodamage,
their Glogau’s photoaging classification was I [10%], II [70%] and
2.72. Their Fitzpatrick skin types were IV [70%] and V [30%] and
whose ages ranged from 40 to 49 with a mean ± SD of 42.40 ±
Clinical Results:

Discussion:
The use of MSC therapies has been limited by the loss of self-
renewal associated with the ex vivo expansion culture, and the
risk of immunogenicity on transplantation due to the conventional
supplementation with fetal bovine serum [FBS] during culture
[12]. A previous study demonstrated that centrifugation of
adipose tissue harvested by liposuction has a negative effect on
tissue architecture and morphology, losing its stem cell content,
as the MSCs is lost in the pellet [13]. In addition, enzymatic
digestion has an impact on the yield and quality of MSC
subsequently isolated from the resulted tissue [14]. There has
been a consensus among fat transfers surgeons that favorable skin
changes can occur down the road following fat transfer [15].
Wrinkles, scars, pores, texture, and other pathologies have been
noted to diminish in areas overlying transplanted fat [16]. Clinical
trials are generally focused on safety and efficiency of a therapy
using a specific type of MSC, without demonstrating which MSC is
the best for each therapy, or even justifying why a specific cell
type was believed to be the best option [17]. In an attempt to
provide insight into this matter with particular attention to skin
aging, we conducted a comparative prospective clinical study
concerning the use of autologous ASCs and BMSCs in the treatment
of the clinical signs of photaging where we put the aforementioned
observations into consideration, and accordingly, the approach
adopted for this research was to inject unprocessed lipoaspirate
in a split-face manner in five of the subjects and in the dorsum of
the right hand of all 10 subjects, as opposed to the bone marrow-
derived bufy coat injected in the left cheek of five of the subjects
and the dorsum of the left hand of all 10 subjects.

Statistical Analysis:
Data were collected, revised, coded and entered to the
Statistical Package for Social Science [IBM SPSS] version 20 and
were presented as mean and standard deviation when
parametric, and median with interquartile range [IQR] when
non-parametric. The comparison between two independent
groups with quantitative data and parametric distribution was
done by using Independent t-test. Also, the comparison between
two paired groups with quantitative data and parametric
distribution was done using Paired t-test, while the comparison
between two paired groups with quantitative data and non-
parametric distribution was done using Wilcoxon Rank test.

Results:
Clinical Results: Ten female participants completed this trial
whose ages ranged from 40 to 49 with a mean ± SD of 42.40 ±
2.72. Their Fitzpatrick skin types were IV [70%] and V [30%] and
their Glogau’s photaging classification was I [10%], II [70%] and
III [20%]. From a clinical standpoint, the scores for photodamage,
according to Griffiths’ photonumeric scale, on both right and left
hands were similar before the treatment. By comparing the pre-
treatment and post-treatment scores for wrinkling on the
dorsum of both hands, all subjects showed significant
improvement from baseline. Comparison of the post-treatment
scores of the right and left hand was found to be statistically non-
significant indicating that the outcome in both hands was very
similar. As for solar lentigines and mottled hyperpigmentation,
there was also a significant improvement in the scores from
baseline to two months post-treatment in both hands. However,
the difference in the post-treatment scores for solar lentigines
between the right and left hand was in favour of the left hand and
was rendered statistically highly significant. The post-treatment
scores for mottled hyperpigmentation in the right and left hand
were compared and the difference was found to be statistically
non-significant. As for the evaluation of photodamage in the face,
only five of the participants had pigmentary lesions on the cheeks
presenting as melasma, solar lentigines or mottled hyperpigmentation.
Not necessarily each one of those signs existed in all of the five participants, for instance melasma was
present in only three of them. The improvement from baseline in
the right cheek was found to be statistically significant for solar
lentigines and mottled hyperpigmentation but not for melasma.
However, in the left cheek, the improvement from baseline was
statistically significant for melasma and highly significant for
solar lentigines. The improvement in the appearance of mottled
hyperpigmentation though, was statistically non-significant.
Comparison of the post-treatment scores for all three lesions in
the right and left cheek was statistically non-significant, thus
indicating that overall both ASCs and BMSCs achieved similar
results.

Histological Assessment:
The specimens were divided into 4 groups. Group I
represents the specimens from the right hand before the
adipose tissue injection. Group III represents the post-adipose
tissue treatment specimens. Group II represents the specimens
from the left hand before the bone marrow-derived bufy coat
injection and lastly, Group IV represents the post-bone marrow
derived bufy coat treatment specimens. Paraffin blocks were
obtained and serial sections of 5 µm thickness were cut and
stained with H&E, Massontrichrome [for collagen], aldehyde
fuchsin [for elastic fibers] and Fontana masson [for melanin]. In
addition, Histometry using Leica Qwin 500 V3 image analyzer
computer system [Wetzlar, Germany] was done for the
specimens stained with special stains. Immunohistochemical
staining was done for characterization of decorinusinga
polyclonal rabbit decorin antibody – Novus – code no. NBP-1-
57923 and the stem cell markers CD10 and CD106 using the
monoclonal mouse antihuman CD10 – Dako – code no. M7308
and the monoclonal mouse antihuman CD106/VCAM-1 – Dako
– code no. M7106, respectively.

FIGURE 5: [A] Figure showing epidermal thickness of a specimen
belonging to Group I pre-treatment [H&E][x100] [B] Figure showing
increased epidermal thickness of the corresponding post-treatment
specimen belonging to Group III [H&E][x100].
Most of the investigations utilizing stem cells have been performed on laboratory animals. There is a shortage of human clinical trials in this regard. A pilot study conducted in 2010 by Kim et al. [18] assessed the anti-wrinkle effect of ASCs in one patient. Processed lipoaspirate cells were injected intradermally for the treatment of peri-orbital wrinkles, and resulted in improvement in general skin texture and wrinkling as evidenced by increased dermal thickness detected by high frequency ultrasonographs using Dermascan-C®. Previous studies indicated that adipose tissue transplantation could improve collagen at the recipient site in addition to increased skin volume [16,18]. Another study performed by Seo et al. [19] assessing the skin rejuvenating effects of human embryonic stem cell-conditioned medium, also revealed similar results, thus concluding that stem cells in general and their secretory factors are promising tools with great prospects in terms of skin regeneration and rejuvenation. Histologically, the adipose tissue-treated group and the bone marrow-treated group both showed an increase in dermal thickness concomitant to the increase in the collagen area percent after the treatment. It is well established that adipose-derived stem cells produce and secrete cytokines/growth factors that antagonize UV-induced photoaging of skin. ADSCs have been shown to improve dermal thickness via the activation of the proliferation of dermal fibroblasts [11]. This has been previously confirmed by Kim et al. in 2009 [20,21] and 2010 [10]. Improved skin regeneration after ASC transplantation in vivo has been shown by Sheng et al. [22]. Furthermore, Zhang et al. in 2014 [23] compiled data that proves ASCs is capable of restoring of the functional capacity of the skin, summarized as follows: [1] ASCs can significantly increase dermal thickness and collagen content of the skin [2] ASCs can decrease the level of advanced glycation end products [3] ASCs can decrease the expression of senescence-associated markers such as superoxide dismutase [SOD] and malondialdehyde [MDA] [4] ASCs can increase the expression level of VEGF and increase the vessel density of the skin. In conclusion, these results demonstrate that ASCs can contribute to the regeneration of the skin and are a potential good candidate for the control and prevention of skin damage caused by glycation in various skin conditions, including wound healing and aging.

The dermal expression of decorin in the specimens of our study was evaluated immunohistochemically showing negative staining for the pre-treatment specimens and positive staining for the post-treatment specimens in our investigated groups. Decorin, the dermatan sulphate proteoglycan, has been demonstrated to co-distribute with and bind to collagen. Decorin interacts with several types of collagen to modulate collagen fibrillogenesis in the body [24]. Thus, it is assumed that the expression of decorin reflects the quality or condition of the collagen fibers and can be used as a marker for qualitative change in collagen fibers [25]. Decorin expression in fibroblasts was found to be upregulated in the metabolically active state during matrix synthesis, since decorin increases collagen type I production and it’s deposition in the extracellular matrix [26,27]. It is also important for regulating both shape and size of the collagen I fibrils, and is therefore vital for matrix organization and the mechanical stability of the extracellular matrix [28].

The morphometric and statistical analysis of the melanin area percent in the bone marrow-treated group revealed a reduction in the melanin area percent, however in the adipose tissue-treated group, it was found to be increased. Additionally, the adipose tissue-treated group showed cut sections of hair follicles and ducts of sweat glands as opposed to the absence of such signs in the bone marrow-treated group. Although both types of MSCs achieved similar clinical results, they had distinctive effects on the histology of the skin at the epidermal level. Uneven distribution of epidermal melanin is the reason behind the clinical manifestation of blotchy hyper-pigmentation characteristic of photoaging [29]. In our adipose tissue-treated subjects, even though there was an increased detection of epidermal melanin but it was evenly distributed along the basal cell layer and so resulted in fading out of the pigmented lesion in contrast to the surrounding skin. Both kinds of MSCs have been shown to contribute to efficient wound healing and improvement of the overlying skin composition and appearance through their transdifferentiation into epithelial cells [30-32]. A study performed by van den Broek et al. [33] in 2012, demonstrated that the skin equivalent model constructed with ASCs upon microscopic examination, exhibited an increase in epidermal thickness as compared to the epidermal thickness promoted by dermal mesenchymal stem cells. The results of our study as regards epidermal thickness concur with the findings obtained by van den Broek, indicating that ASCs in comparison to BMSCs and dermal stem cells are a potent stimulator of epidermal growth.

The differences in the histological findings between the adipose tissue-treated group and the bone marrow-treated group could be explained by a couple of hypothesis concerning the transdifferentiation capacity of the two types of MSCs and their paracrine capabilities. It has been established that gender differences regarding the paracrine capacity of ADSCs exist, and therefore, it can be postulated that similar differences exist between MSCs from different sources. ASCs have been shown to alleviate the condition of photodamaged skin via targeting dermal fibroblast activity. Transforming growth factor beta [TGF-β] appears to be the most potent stimulator of collagen remodeling by fibroblasts [34,35]. The TGF-β/Smad pathway is the major regulator of the synthesis of several components of the extracellular matrix, including type I and type III collagen. Previous studies suggested that a number of signaling pathways govern the production of collagen type I, one of which is the highly conserved Wnt/β-catenin pathway [36, 37, 38]. Wnt/β-catenin signaling integrates signals including TGF-β and fibroblast growth factor [FGF], among numerous signaling pathways, to mediate a variety of cellular activities. Thus, the activity of wnt3a and β-catenin in the Wnt/β-catenin signaling pathway is important to TGF-β2 for the production of collagen type I. UVB irradiation reduces the expression of wnt3α and β-catenin, and consequently reduces that of TGF-β2 and collagen type I. LiXuan and colleagues [11] concluded that ADSC-CM does accelerate the expression of wnt3α and β-catenin in treated photoaging HDFs, and that the expression of TGF-β2 has a positive correlation with the expression of wnt3α and β-catenin. Therefore it is believed that one of the mechanisms by which ASCs treatment improves photoaging skin is the rescued activity of Wnt/β-catenin signaling.

Moreover, activation of Wnt signaling in keratinocytes influences the behavior of melanocytes, nerves, and fibroblasts through activating signals that control pigmentation and nerve fiber growth [39, 40]. Each melanocyte at the basal layer of the...
epidermis is functionally connected to underlying fibroblasts in the dermis and to keratinocytes in the overlying epidermis. Those three types of cells are highly interactive and communicate with each other via secreted factors and their receptors and via cell/cell contact to regulate the function and phenotype of the skin. Inhibition of Wnt signaling in melanocytes dramatically inhibits the melanogenenic pathway [41]. Increased expression of Wnt inhibitory factor-1 (WIF-1) gene in keratinocytes significantly reduced the expression of tyrosinase in melanocytes. The ratio of [TYRP-1] tyrosinase-related protein-1 positive cells also decreased [42].

ASCs have been shown to increase melanocyte proliferation and migration, while reducing differentiation in a study conducted by Kim et al. in 2012 where melanocytes were co-cultured with ASCs and keratinocytes individually, and compared to melanocyte monolculture. Their results revealed an increase in melanocyte numbers with an increase in the proportion of less pigmented melanocytes that were sustained for a longer period of time in the presence of ASCs [43]. Furthermore, recent studies suggest that extrafollicular dermal melanocyte stem cells do persist after birth and give rise to migratory melanocyte precursors when replacements are needed for epidermal melanocytes within the basal layer[44]. It has been reported that melanocyte stem cells express receptors for Wnt signaling pathway on their surface[45]. Wnt signaling regulates the quiescence, expansion and differentiation of melanocyte stem cells[44]. Given the presence of a heterogeneous population of stem and progenitor cells within the adipose tissue stroma, activation of melanocyte stem cells within the overlying skin after the subcutaneous injection of adipose tissue is likely [46]. Sweat-gland development requires the synergetic interaction of stem cells, growth factors, and matrix metalloproteinase. In human fetal skin, epidermal stem cells serve as the stem-cell source for sweat gland development. Growth factors, cytokines, and extracellular matrix components are all required for these cells to proliferate and differentiate into mature epidermal cells. In particular, epidermal growth factors may serve as autocrine or paracrine modulators that signal epidermal cells to form sweat-gland cell clusters [47, 48]. Matrix metalloproteinase also help sweat gland bud formation and migration from epidermis to dermis. However, adult skins can no longer naturally regenerate sweat glands in this way. Moreover, it lacks a postdamage regeneration process. Therefore, sweat gland regeneration must be stimulated by exogenous stem cell therapy [49]. ASCs’ capacity to transdifferentiate into epidermal stem cells is a concept that has been investigated in a study performed by Derby et al. [32] in 2013, on the basis of green fluorescent protein (GFP) signals and co-staining with specific primary antibodies, in an attempt to potentially improve understanding of fat grafting’s impact on skin rejuvenation. They offered suggestive evidence of the presence of GFP+ cells, within the overlying dermal architecture, that co-expressed the epithelial stem cell marker p63, 8 weeks after whole fat engraftment into the subdermal plane of para-scapular skin flaps. Given the results of our study, we can deduce that ASCs are superior to BMSCs in terms of their transdifferentiation into epithelial cells.

As regards the elastin area percent in the post-treatment specimens of our study, an increase was detected in both groups. This finding conforms to findings of another study conducted by Tian et al. [50] to investigate the effect of stem cells on the expression of elastin in smooth muscle cells for the treatment of abdominal aortic aneurysm, also demonstrated that both ADSCs and BMSCs upregulated the gene and protein expression of elastin, and resulted in a decrease in the activity of MMP-2. These data together with the results of our study prove that stem cells are capable of normalizing the dermal features of photoaged skin. Taken all together, the promising results of our study render stem cells and their cell-derived biomolecules a promising clinically applied line of anti-aging remedies.

Conclusion:
In light of our results, stem cells in general and mesenchymal stem cells in particular, have great therapeutic potential regarding the reversal of the histological-functional damage brought upon by a multitude of age-accelerating factors especially solar radiation. From a dermatological aspect, ASCs have an added advantage as they localize in the hypo-dermal compartment of the skin, and could presumably be innately programmed to revive the function and vitality of the skin with all of its constituents, more so than mesenchymal stem cells derived from other tissues.

References:


