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Combination of Low-Level Laser Therapy and Autologous Exosome Therapy in Hair Growth; Case Series

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Abstract **Case Report**

Regenerative medicine methods in the beauty field have quickly attracted the attention of many doctors and scientists in recent years. These methods include cell therapy, exosome therapy, and laser therapy. Based on many studies, exosomes, play a decisive role in the body's regeneration process. On the other hand, many articles confirm that laser therapy with a very important mechanism is very effective in enhancing hair growth. In this article we want to show you examples of the very successful effects of the simultaneous application of laser therapy and autologous exosome therapy on volunteers who have agreed to use these methods to hair growth. After autologous exosome preparation, the concentration is 0.15 to 0.3 ml per cm^2 of skin for hair growth. The total number of injections for hair growth is 5 ml. In Hair growth, the treatment area was getting thinner and thinner or gradual to moderate hair loss on the top of the head, and hair growth injection was done by 30-27G, 4mm to 6mm needle. The treatment interval for hair growth is 1 to 2 sessions, monthly. In each session, immediately after autologous exosome injection, we performed laser therapy, 1 joule per cm^2, output power of 200 mW, and laser wavelengths of 630 nm and 780 nm. It appears to be due to the synergistic effect between these two approaches, i.e. the use of autologous exosomes and laser therapy; look out for the combination of these two methods in the field of beauty and other therapeutic applications in the future.

Keywords: Exosome, Extracellular Vesicle, Dermatology, Aesthetic, Laser Therapy, Hair Growth.

INTRODUCTION

Hair growth are the two most popular fields in medicine [1-3], and specially in cosmetology and dermatology [4]. Many studies use PRP and, low-intensity laser therapy for hair growth [5, 6]. Therefore, the continued desire for anti-aging and hair growth interventions has fueled the demand and rapid popularity of non-surgical cosmetic procedures. Over the past decade, exosomes have attracted particular interest as a topical and injectable solution due to their described regenerative properties and their potential influence on wound healing, scar revision, and hair grow [6]. Exosomes are cell-derived nano-sized vesicles that carry various biomolecules, including proteins, nucleic acids, and lipids, capable of communicating with neighboring or distant cells. Exosome technology, although still in its infancy, is currently being exploited in regenerative cosmetic medicine due to its multifaceted role in combating the root causes of aging and improving overall homeostasis. Exosomes can act as intercellular messengers to influence neighboring cells, while retaining certain biological properties of the parent cell. Exosomes can regulate essential cellular processes, such as proliferation, differentiation, migration and cell death, which vary depending on the origin of the exosome, physiological and pathological state and even the release of cells at the correct location [7-9].

In hair follicles, exosome-mediated intercellular communication and information transfer are necessary to maintain cellular functions. MSC-derived

exosomes can be safely used as topical treatments without adverse effects. These, demonstrate the potential of exosomes as therapeutic agents, cosmetic ingredients or for other biological uses [10].

Although one of the most studied exosome derivatives is derived from adipose tissue, it is important to note that each tissue or cell of origin may exert specific effects by specific mechanisms through its own mechanism. One commonly reported exosome target is manipulation of the TGF-β (transforming growth factor beta) superfamily, which is essential for cell differentiation, proliferation, and apoptosis [11-13].

On the other hand, there are many studies on the effects of low-intensity laser therapy on hair growth. The mechanism of low-intensity laser therapy is very beneficial to cell therapy methods, including exosome and stem cell therapy [13].

Low-level laser therapy technique without thermal reaction can increase micro and macro blood circulation. Low-energy lasers can inhibit nitric oxide activity, leading to more cellular oxidative activity. Evidence suggests that low laser therapy acts on mitochondria and may alter cellular metabolism through photodissociation of nitric oxide inhibiting cytochrome c oxidase in complex IV in the mitochondrial respiratory chain, which increases ATP production, modulates reactive oxygen species, and induces transcription factors such as nuclear factor kappa B and hypoxia-inducible factor 1. These transcription factors in turn induce protein synthesis and induce other effects, such as cell proliferation and migration, changes in levels of cytokines, growth factors and inflammatory mediators, and increased tissue oxygenation [3, 14-17].

Laser therapy is hypothesized to stimulate anagen cycle into telogen hair follicles, prolong the duration of the anagen phase, increase the rate of proliferation in active anagen hair follicles, and prevent catagen development in early state [16].

This case series study aims to highlight the current and emerging landscape of exosome and low-level laser therapy in aesthetic medicine, including hair restoration.

MATERIAL AND METHODS

Autologous Exosome

In the presence of a cellular density of 80%, the human adipose derived mesenchymal stem cells-conditioned media will be harvested every 48 h. To sterilize the supernatants and remove larger extracellular vesicles (EVs), the conditioned media will be passed through 0.22 mm filter membranes and centrifuged at 700 g for 10 min to remove cell debris. Another round of centrifugation will be performed at 9000 g for 30 min, and the supernatant will be collected again. Exosomes will be isolated by ExoEasy Maxi kit (76064; Qiagen) 3 and re-suspended in PBS. Pellets will be washed with 0.9% NaCl and re-precipitated at 100,000 g at for 2 h. The obtained pellets will be dissolved in 0.9% NaCl to a final concentration of 1 unit EVs per 1 ml. The characterization of exosomes will be achieved by measuring expression of exosome-specific markers CD81, CD9, nex1 and CD63 by Western blot analysis and particle size by NanoSight analysis. The concentration of exosome-enriched EVs will be determined by analyzing protein concentration using the Bradford protein quantitation assay kit (Zist Tolid Razi) with BSA as a standard. Exosome morphology will be assessed using a transmission electron microscope.

Human adipocyte derived mesenchymal stromal cells (hADSCs) passages 3–6 was maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% Human AB serum (mycoplasma, CMV, HBV, HCV and HIV tests performed by PCR), 100 U/ml penicillin and 100 µg/ml streptomycin and incubated at 37°C in 5% CO2. Cells were grown to 70%–80% confluency, washed three times with PBS and incubated for 48 h in serum-free media (low-glucose DMEM/F12). In the presence of a cellular density of 80%, the hADSCs-conditioned media were harvested every 48 h. To sterilize the supernatants and remove larger EVs, the conditioned media were passed through 0.22 mm filter membranes and centrifuged at 700 g for 10 min at 4°C to remove cell debris. Another round of centrifugation was performed at 9000 g at 4°C for 30 min, and the supernatant was collected again. Exosomes were isolated by ExoEasy Maxi kit (76064; Qiagen) and re-suspended in phosphate-buffered saline (PBS). Residual polyethylene glycol and soluble proteins that might have been coprecipitated were removed, pellets were washed with 0.9% NaCl and reprecipitated at 100,000 g at 4°C for 2 h. The obtained pellets were then dissolved in 0.9% NaCl to a final concentration of 1 unit EVs per 1 ml and stored as 1 ml aliquots at −80 C until usage. The characterization of exosomes was achieved by measuring expression of exosome-specific markers CD81, CD9, nex1 and CD63 by Western blot analysis and particle size by NanoSight analysis (platforms SLM 20). The concentration of exosome-enriched EVs was determined by analyzing protein concentration using the Bradford protein quantitation assay kit (Zist Tolid Razi) with BSA as a standard.

Injection Method

After autologous exosome preparation, the concentration is 0.15 to 0.3 ml per cm^2 of skin for hair growth. The total number of injections for hair growth is 5 ml. In Hair growth, the treatment area was getting thinner and thinner or gradual to moderate hair loss on the top of the head, and hair growth injection was done by 30-27G, 4mm to 6mm needle. The treatment interval for hair growth is 1 to 2 sessions repeated monthly. In each session, immediately after autologous exosome

injection, we performed laser therapy, 1 joule per cm^2, output power of 200 mW, and laser wavelengths of 630 nm and 780 nm.

RESULTS

Figure 1: The patient is a 31-year-old, female, with high stress and nervous tension, taking minoxidil for 6 months and 5 sessions of mesotherapy before the doctor was no longer effective. After consulting him and performing the necessary tests, the blood tests were normal, exosome and laser therapy were started for him at the same time, and the minoxidil was tapered, the result will be seen after 60 days

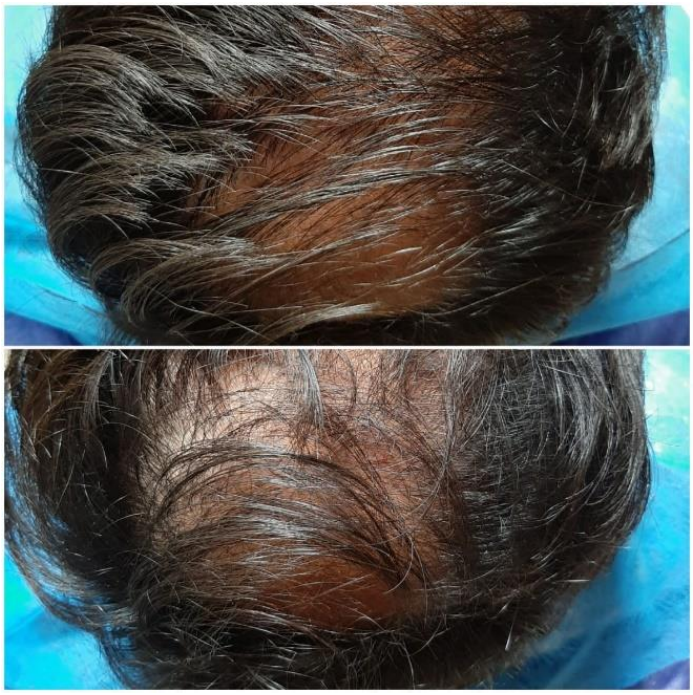

Figure 2: The patient is a 22-year-old man with a family history of hair loss, a relatively unsuccessful transplant in the area of the hair growth line, 5 sessions of mesotherapy and the use of minoxidil, which failed 60 days after the patient's lack of consent; After the initial tests, exosome and laser therapy started for him at the same time, and you can see the result 2 months later

Figure 3: A 16-year-old male with a history of severe and genetic hair loss, who was treated by another doctor for PRP injections and it was not effective. The patient was again on treatment with vitamin D stimulation, which was not successful. After 4 months from the last treatment and failure of previous treatments; and after the patient's lack of consent; Combined treatment of exosome and laser treatment was started, and satisfactory results are observed after 2 sessions with an interval of 30 days

Figure 4: A 35-year-old female, 2 sessions of mesotherapy and PRP injection, which failed. After 60 days the patient's lack of consent; exosome and laser therapy, and you can see the result 2 months later

Figure 5: A 35-year-old man with a family history of hair loss, a relatively unsuccessful transplant, 3 sessions of mesotherapy and PRP injection, and three months use of minoxidil, which all failed. After 60 days the patient's lack of consent; exosome and laser therapy, and you can see the result 2 months later

Figure 6: A 33-year-old male patient with a history of itching, dryness and scalp inflammation, hair loss; Thinning of hair, which was normal for thyroid, vitamin D and CBC tests, 3 courses of 5% urea shampoo along with PRP injection were not effective. Six months after the last treatment, exosome along with laser therapy was performed for them, and the result after one treatment session can be seen 30 days later

Figure 7: A 35-year-old female, with a history of severe hair loss, the requested tests were normal. exosome injection and laser therapy were performed for her at the same time, you will see the result after two months

DISCUSSION

As demonstrated by many studies, low-intensity laser therapy can regulate various pathways, which are essential for stress response, cellular functioning, and survival rate of stem cells. Low-level laser therapy can also promote antioxidant capacity, mitochondrial biogenesis, and ATP production in cells. In addition, it can improve the proliferation and migration of stem cells and stem cell products such as exosomes [14, 18, 19]. According to the effects of low-level laser therapy in regulating the function of stem cells and extracellular vesicles, it appears that combining stem cell treatment and low-level laser therapy may yield effective results. more effective and stable in treatment [20]. diseases. Low-level laser therapy can lead to improved ATP levels, biogenesis, and mitochondrial function, reducing oxidative stress factors essential for stem cell survival and function. Ultimately, all these conditions may increase the effectiveness of stem cell therapy and exosome therapy [21].

CONCLUSION

We propose a treatment method that combines stem cell secretion and extracellular vesicles, exosomes; to obtain more useful and stable results during treatment. Therefore, the combination of low-level laser therapy and exosome therapy as a new medical approach to modulate oxidative stress and focus on mitochondrial function may provide more effective results in combined aesthetics and perhaps in disease treatment.

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