ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 04 | April - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ADULT STEM CELL RELEASED MOLECULES IN COMBINATION WITH MICRONEEDLING RESTORE HAIR GROWTH

Clinical Research

Michael Ryan Clinics, Dubai & Manchester, UK
Greg Maguire* NeoGenesis Inc San Diego, CA 92121 USA. *Corresponding Author

ABSTRACT

Introduction: Alopecia is a chronic dermatological disorder affecting millions of people, in which people lose some or all of the hair on their head. Although alopecia has many forms, all are characterized as a chronic inflammatory disease that affects the hair follicles. Alopecia often has psychological consequences, including high levels of anxiety and depression.
Case presentation: We report hair regrowth in 12 of 13 patients with alopecia treated with adult stem cell released molecules in combination with micro needling.
Conclusion: Adult stem cell released molecules in combination with micro needling is an efficacious, safe, and affordable treatment for alopecia.

KEYWORDS

Alopecia; Micro needling; Stem Cell Released Molecules; Hair Growth.

INTRODUCTION

Multiple factors contribute to hair loss, including aging, heredity, hormones, environmental exposure, stress, medications, and nutrition. Hair growth is a highly complicated stem cell-based process and serves as a model for general stem cell function where stem cells in the follicle interact with stem cells and fibroblasts in the surrounding skin (Schmidt-Ullrich R and Paus, 2005; Weir and Garza, 2020). The dermal papilla (DP), a cluster of specialized fibroblasts in the follicle, secretes diffusible proteins packaged into exosomes that regulate the growth and activity of the various cells in the follicle, thereby playing a key role within the follicle in the regulation of hair cycling and growth (Zhou et al, 2018). Extracellular vesicles (EVs), including exosomes, are the carriers for the distribution of morphogens and growth and differentiation factors (Riazifar et al, 2017), and can pack thousands of proteins into one exosome, delivering that collective cargo to one target in the same time and space (Maguire, 2016). The DP is encapsulated by an overlying matrix of epithelial cells, and growth factors from DP are believed to cause epithelial cells to proliferate and differentiate to produce hair shafts during the anagen phase. In addition to hair loss, Nishimura *et al* (2005) have demonstrated that hair graying is caused by defective self-maintenance of melanocyte stem cells in the follicle. Despite the efforts of scientists in seeking effective therapeutic agents, only a few marginally effective Food and Drug Administration (FDA)-approved medications are available for alopecia patients. The mostly widely prescribed drugs for alopecia are Finasteride and minoxidil, either as monotherapy or in combination. Although finasteride has been found to enhance hair growth, oral finasteride often causes reduced libido, impotence, and sexual dysfunction (Fertig et al, 2017). Further, this treatment is only applicable to male patients with AGA given the highly teratogenic effects of finasteride (Kawashima et al, 2004). Topical minoxidil (2%) is the only treatment for female patients with AGA, and this has lower efficacy than the 5% minoxidil preparation that is available for male patients (Olsen et al, 2002), thus resulting in disappointing outcomes. Furthermore, minoxidil can affect the heart and blood pressure if absorbed excessively through the skin (Goren and Naccarato, 2018).

While adipose derived mesenchymal stem cells (ADSC) molecules have been found to regrow hair in a randomized, double blind, placebo controlled clinical trial (Tak et al, 2020), the effectiveness of these molecules in a real-world clinical setting has not been reported. Here we report in a clinical setting the use of stem cell released molecules combined with micro needling is a safe and efficacious means to regrow hair in those patients with alopecia.

Case Report

Subjects

A total of thirteen subjects were enrolled in our case studies. Participants ranged in age from 28 to 64 years. All subjects were recruited for the study and treated by Dr. Michael Ryan, who is a practicing trichologist at the Dubai Hair Clinic. Men and women eligible for inclusion in the trial were in good general health with no evidence of systemic illnesses (e.g., cardiac, psychiatric, or scalp disease). Patients known to be hypersensitive to minoxidil were excluded, as were patients who concomitantly used hair restorers or systemic drugs (steroids, cytotoxic agents, vasodilators, antihypertensive agents, anticonvulsant drugs, β-adrenergic receptor blockers, diuretics, or any of the following specific agents: spironolactone, cimetidine, diazoxide, cyclosporine, or ketoconazole).

Procedure

This was a 24 - week trial conducted at 1 investigative site in the UAE. The protocol and informed consent form were approved by the Dubai Health Authority guidelines, and written informed consent was obtained from each patient before enrollment in the trial. The test solution with a dose of 1mL of assigned solution was applied to the frontoparietal and vertex areas of the scalp for 24 weeks, daily both morning and evening. In addition, on visits to the trichologist occurred bi-weekly, at which time the test solution was applied and then the scalp was microneedle. Microneedling was performed using an Eclipse Micropen Elite, and the micro needling procedure included multi directional passes (lateral, horizontal and 45 degrees to the parting) of the device over the treated area at a depth of 0.75mm – 1.5mm, prior to application of the test solution. Variable depths of the micropen were required in order to produce erythema at the target site, some pin point bleeding was noticeable. Upon erythema being noticeable, 1mL test solution was then applied via a no-needle syringe to the target area and the micro pen procedure was repeated with a targeted approach. After the baseline visit (week 1), patients returned to the clinic for efficacy and safety evaluations every 6 weeks through week 12, then every 6 weeks through the end of the 24 -week trial.

Hair counts were obtained by using a Firefly Pro 330T derma scope using, a 1 cm2 target evaluation area in the thinning vertex scalp, defined by an area consistently measured at 8cm and 20cm from the center of the subject's eyebrows to the center of the vertex. Images were captured at baseline and weeks 1, 6, 12, and 24, were calculated by a computer program (TrichosciencePro V 1.7SE microscopic evaluation software). This analysis was performed by a trained trichologist. The resulting hair counts and hair thicknesses per square centimeter were used to calculate mean change from baseline.

Product Ingredients

Human Stem Cell Conditioned Media, Human Fibroblast Conditioned Media, Water, Glycerin, Larix Europaea Wood Extract, Camellia Sinensis Leaf Extract, Santalum Acuminatum Fruit Extract, Citrus Glauca Fruit Extract, Acacia Victoriae Fruit Extract, Trifolium Pratense (Clover) Flower Extract, Zinc Chloride, Glycine, Hydroxyethylcellulose, Dehydroacetic Acid, Benzyl Alcohol, Lactic Acid.

Choice of Stem Cell Types for Deriving the Stem Cell Released Molecules

Not all adult stem cells are alike. Elly Tanaka's lab (Kragl et al, 2009) found that adult stem cells are tissue specific, meaning that adult stem cells from a specific tissue are optimal for repairing and regenerating that specific tissue. In these studies, we used adult stem cells to make and release the molecules used in the test product that are derived from the hair follicle and the skin surrounding the hair follicle. Cell types used for therapeutic development must be chosen carefully for the sake

of safety and efficacy. Maguire (2019; 2021) and Conese et al (2020) have reviewed why ADSCs are the preferred stem cell type for developing therapeutics, especially for skin conditions. They review why other stem cells types, notably bone marrow mesenchymal stem cells (BMSCs), are a poor choice for many therapeutic applications. For example, many lines of evidence from *in vitro* and *in vivo* studies, including those in human BMSC transplants, find that BMSCs and their exosomes are carcinogenic and metastatic (Qi et a;, 2017; Luo et al, 2020; Huang et al, 2020), owing to a number of factors, including the fusion of BMSCs with cancer cells (Terada et al, 2002) or by the education of BMSCs by cancer cells that yields a cancerous phenotype in the BMSC (Nakata et al, 2017; Sai et al, 2019). Indeed, BMSCs transplants in humans routinely increase the risk of cancer and death in the recipient (Maguire, 2019B; Zhao et al, 2019). Further, exosomal transfer of miRNAs from the bone marrow mesenchymal stem cells may promote breast cancer cell dormancy, such that a state of breast cancer may lay in wait for an appropriate signal (Ono et al, 2014). These oncogenic factors have not been found in ADSCs or their exosomes (Maguire, 2019), including the ADSCs and their exosomes from cancer patients (García-Contreras et al, 2014).

RESULTS

Significant hair growth was observed in all but one patients treated. Although there were small changes to pigmentation color in some candidate, pigment changes were unmeasurable in this study.

Terminal Hair Counts

In 12 of 13 patients patients significant hair growth was achieved by the combined use of microneedling and topical application of stem cell released molecules (see Table 1).

Table 1. Individual counts of terminal hair units per given area in the scalp of patients who were treated with a combination of micro needling and stem cell released molecules. Values are presented for before treatment and after treatment.

Subject	Sex	Age	Duration Of Treatment Weeks	Hair Density Before (sq-cm)	Hair Density After (sq-cm)
Ali	M	41	12	140	177
Nida	F	32	24	120	161
Aswain	M	39	12	107	140
Sal	M	45	12	102	248
Has	M	39	12	107	96
San	M	34	12	178	200
Jo	M	28	24	96	126
Sand	M	42	12	113	140
Nak	M	48	24	133	196
Son	M	49	24	42	70
Naz	F	44	12	81	111
Rav	F	62	24	117	285
Man	M	64	24	78	93

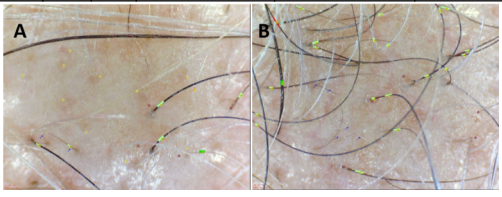

Figure 1. Example of hair count measurements, A. before treatment and, B. after treatment. Green colored areas are computer determined, and pseudo-color coded terminal hairs as distinguished from vellus hairs.

Thickness (Diameter) of Individual Terminal Hairs

In 10 of 13 patients significant hair thickness (diameter) was achieved by the combined use of microneedling and topical application of stem cell released molecules (see Table 2).

Table 2. Individual counts of terminal hair unit thickness (diameter) in the scalp of patients who were treated with a combination of micro needling and stem cell released molecules. Values are presented for before treatment and after treatment.

Subject	Sex	Age	Duration Of Treatment Weeks	Hair Thickness Before (sq-cm)	Hair Thickness After (sq-cm)
Ali	M	41	12	>30 µm): 87% (64.2 per sq.cm)	>30 µm): 82% (75.3 per sq.cm)
Nida	F	32	24	(>35 µm): 76% (81.5 per sq.cm)	(>35 µm): 88% (68.5 per sq.cm)
Aswain	M	39	12	(>30 µm): 85% (85.2 per sq.cm)	(>30 µm): 79% (96.3 per sq.cm)
Sal	M	45	12	(>30 µm): 82% (33.3 per sq.cm)	(>30 µm): 88% (34.5 per sq.cm)
Has	M	39	12	(>30 µm): 85% (81.5 per sq.cm)	(>30 µm): 92% (85.2 per sq.cm)
San	M	34	12	(>30 µm): 91% (107.4 per sq.cm)	(>30 µm): 86% (88.9 per sq.cm)
Jo	M	28	24	(>35 µm): 81% (63.0 per sq.cm)	(>35 µm): 84% (100.0 per sq.cm)
Sand	M	42	12	(>30 µm): 95% (98.1 per sq.cm)	(>30 µm): 87% (79.3 per sq.cm)
Nak	M	48	24	(>30 µm): 91% (107.4 per sq.cm)	(>30 µm): 88% (159.3 per sq.cm)
Son	M	49	24	(>30 µm): 87% (15.5 per sq.cm)	(>30 µm): 97% (36.3 per sq.cm)
Naz	F	44	12	(>30 µm): 95% (77.8 per sq.cm)	(>30 µm): 100% (111.1 per sq.cm)
Rav	F	62	24	(>35 µm): 88% (25.9 per sq.cm)	(>35 µm): 71% (31.5 per sq.cm)
Man	M	64	24	(>30 µm): 92% (44.4 per sq.cm)	(>30 µm): 88% (77.8 per sq.cm)

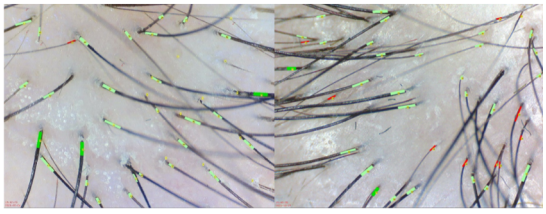

Figure 2. Example photo used to measure hair unit thickness, A. Before and B. After treatment.

DISCUSSION

The secretome derived from ADSCs and fibroblasts include PDGF, FGFs, HGF, VEGF, IGF binding protein precursors, and fibronectin (An et al, 2021). PDGF has been found to induce and maintain the anagen phase in the hair cycle in a mouse model, and HGF facilitates hair follicle elongation (Lee et al, 2001; Yomita et al, 2006). VEGF increases hair growth and size of the follicle by vascularization of the follicle (Yano et al, 2001), and IGF-1 improves the migration, survival, and proliferation of hair follicle cells (Gentile and Garcovich, 2019). FGFs secreted from fibroblasts also promote hair growth by inducing the anagen phase in resting hair follicles (Lin et al, 2015). Apoptosis is a normal process in the hair cycle, and mesenchymal stem cells help to clear apoptotic cells, following which they release PGE2 that decreases inflammation (Zhang et al, 2019). Thus, EVs from ADSCs, which have been involved in apoptotic clearance, may contain PGE2 and reduce inflammation in the hair follicle, thus augmenting hair growth. Recent studies in a mouse model of hair loss found that epithelial stem cells in the follicle escape from their stem cell niche into the dermis in aged mice, thus contributing to the miniaturization of the hair follicle (Zhang et al, 2021). Because stem cells secrete factors that maintain the stem cell niche (Lee et al, 2016), a plausible partial explanation for how the secretome of ADSCs facilitates hair growth is that the niche is maintained and inhibits stem cell escape from the follicle. In general, during the cyclic quiescence and activation of hair growth, hair follicle stem cells in the resting phase constantly "sum" the input of activators and inhibitors, and when total activators become dominant, the follicle enters growth phase (Lei and Chuong, 2016). The molecules used in this study help to drive the sum of signaling to that of activation.

In conclusion, we've provided evidence in a real-world, clinical setting that a combination of micro needling and stem cell released molecules (S2RM) induces a significant increase in terminal hair counts and hair thickness that is objectively measured using state of the art clinical imaging, and that is subjectively observed by the clinician and patient alike as significant hair growth.

REFERENCES

1. An YH et al (2021). High-Efficient Production of Adipose-Derived Stem Cell (ADSC)

Secretome Through Maturation Process and Its Non-scarring Wound Healing Applications. Frontiers in bioengineering and biotechnology, 9, 681501. https://doi.org/10. 3389/fbioe.2021.681501
2. Botchkarev V.A and Kishimoto J. (2003) Molecular control of epithelial-mesenchymal interactions during hair follicle cycling.J Investig Dermatol Symp Proc. 8: 46-55
3. Conese M et al (2020) The Role of Adipose-Derived Stem Cells, Dermal Regenerative Templates, and Platelet-Rich Plasma in Tissue Engineering-Based Treatments of Chronic Skin Wounds. Stem Cell Intl, V 2020 |Article ID 7056261 | 17 pages.
4. Fertig RM, Gamret AC, Darwin E, et al (2017) Sexual side effects of 5-α-reductase inhibitors finasteride and dutasteride: a comprehensive review. Dermatol Online J. 23:13030/qt24k8q743.
5. García-Contreras M, Vera-Donoso CD, Hernández-Andreu JM, García-Verdugo JM, Oltra E (2014) Therapeutic Potential of Human Adipose-Derived Stem Cells (ADSCs) from Cancer Patients: A Pilot Study. PLoS ONE 9(11): e113288. https://doi.org/ 10. 1371/journal.pone.0113288.
6. Gentile P, Garcovich S (2019) Advances in Regenerative Stem Cell Therapy in Androgenic Alopecia and Hair Loss: Wnt pathway, Growth-Factor, and Mesenchymal Stem Cell Signaling Impact Analysis on Cell Growth and Hair Follicle Development. Cells. 8(5):466.
7. Goren A, Naccarato T (2018) Minoxidil in the treatment of androgenetic alopecia. Dermatol Ther. 2018;31:e12686.
8. Huang Y, Wei Liu, Bing He, Lei Wang, Fucheng Zhang, Hao Shu, Luning Sun,
9. Exosomes derived from bone marrow mesenchymal stem cells promote osteosarcoma development by activating oncogenic autophagy,
10. Journal of Bone Oncology, 21: 100280.
11. Kawashima M, Hayashi N, Igarashi A, et al (2004) Finasteride in the treatment of Japanese men with male pattern hair loss. Eur J Dermatol.14:247-254.
12. Kragl et al (2009) Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature 460, 60–65.
13. Lee YR et al (2001) Hepatocyte growth factor (HGF) activator expressed in hair follicles is involved in in vitro HGF-dependent hair follicle elongation. J Dermatol Sci. 2001;25:156-163.
14. Lee JY, Chen JY, Shaw JL, Chang KT (2016) Maintenance of Stem Cell Niche Integrity by a Novel Activator of Integrin Signaling. PLoS Genet 12(5): e1006043. https://doi.org/10.1371/journal.pgen.1006043.
15. Lei M, Chuong CM (2016) Stem cells. Aging, alopecia, and stem cells. Science. 351 (6273):559-60.
16. Luo et al (2020) Mesenchymal Stem Cell-Secreted Exosome Promotes Chemoresis tance in Breast Cancer via Enhancing miR-21-5p-Mediated S100A6 Expression. Molecular Therapy: Oncolytics, 19:283-293.
17. Maguire G (2019) The Safe and Efficacious Use of Secretome From Fibroblasts and Adipose-derived (but not Bone Marrow-derived) Mesenchymal Stem Cells for Skin Therapeutics. J Clin Aesthet Dermatol. 12(8):E57-E69.
18. Maguire G (2019) Transplanted Stem Cells Survive A Long Time – Do They Make You Sick? J. Roy. Soc. Med. 112(10):412-414.
19. Maguire G (2016) Exosomes: smart nanospheres for drug delivery naturally produced by stem cells. In: Fabrication and Self-Assembly of Nanobiomaterials, Editors: Alexander Grumezescu, Elsevier.
20. National Center for Complementary and Integrative Health: Complementary, Alternative, or Integrative Health: What's in a Name? 2016. https://nccih.nih.gov/sites/nccam.nih.gov/files/Whats_In_A_Name_06–16–2016.pdf.
21. Nakata R et al (2017) Contribution of neuroblastoma-derived exosomes to the production of pro-tumorigenic signals by bone marrow mesenchymal stromal cells. J Extracell Vesicles. 6(1):1332941.
22. Nishimura EK, Granter SR, Fisher DE (2005) Mechanisms of hair graying: Incomplete melanocyte stem cell maintenance in the niche. Science. 307:720–4.
23. Olsen EA, Dunlap FE, Funicella T, et al (2002) A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. J Am Acad Dermatol. 47:377-385.
24. Ono M et al (2014) Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Science Signaling, Vol. 7, Issue 332, pp. ra63.
25. Qi J, Zhou Y, Jiao Z, Wang X, Zhao Y, Li Y, Chen H, Yang L, Zhu H, Li Y. Exosomes Derived from Human Bone Marrow Mesenchymal Stem Cells Promote Tumor Growth Through Hedgehog Signaling Pathway. Cell Physiol Biochem. 2017;42(6):2242-2254.
26. Lin WH et al (20-15) Fibroblast Growth Factors Stimulate Hair Growth through β-Catenin and Shh Expression in C57BL/6 Mice. Biomed Res Intl, Volume 2015 |Article ID 730139
27. Riazifar M et al (2017) Stem Cell Extracellular Vesicles: Extended Messages of Regeneration, Annual Review of Pharmacology and Toxicology 57:1, 125-154.
28. Sai, B., Dai, Y., Fan, S. et al. (2019) Cancer-educated mesenchymal stem cells promote the survival of cancer cells at primary and distant metastatic sites via the expansion of bone marrow-derived-PMN-MDSCs. Cell Death Dis 10, 941.
29. Schmidt-Ullrich R and Paus R (2005) Molecular principles of hair follicle induction and morphogenesis. Bioessays. 27(3):247-61.
30. Tak YJ et al (2020) A randomized, double─ blind, vehicle─ controlled clinical study of hair regeneration using adipose─ derived stem cell constituent extract in androgenetic alopecia. Stem Cells Trans. Med., 9:839–849.
31. Terada, N., Hamazaki, T., Oka, M. et al. (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416, 542–545 (2002). https://doi.org/10.1038/nature730.
32. Tomita Y, Akiyama M, Shimizu H (2006) PDGF isoforms induce and maintain anagen phase of murine hair follicles. J Dermatol Sci. 2006;43:105-115.
33. Wier EM and Garza LA (2020). Through the lens of hair follicle neogenesis, a new focus on mechanisms of skin regeneration after wounding. Seminars in cell & developmental biology, 100, 122–129.
34. Zhang, C., Wang, D., Wang, J. et al (2021) Escape of hair follicle stem cells causes stem cell exhaustion during aging. Nat Aging. https://doi.org/10.1038/s43587-021-00103-w
35. Zhang, Z., Huang, S., Wu, S., Qi, J., Li, W., Liu, S., Cong, Y., Chen, H., Lu, L., Shi, S., Wang, D., Chen, W., & Sun, L. (2019). Clearance of apoptotic cells by mesenchymal stem cells contributes to immunosuppression via PGE2. EBioMedicine, 45, 341–350.
36. Zhao, L., Chen, S., Yang, P. et al. The role of mesenchymal stem cells in hematopoietic stem cell transplantation: prevention and treatment of graft-versus-host disease. Stem Cell Res Ther 10, 182 (2019).
37. Zhou L et al (2018) Regulation of hair follicle development by exosomes derived from dermal papilla cells, Biochemical and Biophysical Research Communications, 500: 325-332