Coil Phenomenon that Occurs in Hair Loss Patients after Autologous Micro Transplantation and the Effect of Targeted Exosomes on Hair Growth

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〈Abstract〉

Recently, as a method of hair loss treatment, a non-surgical method of applying the technology of isolating a patient's specific autologous progenitor cells after biopsy and using it by injecting isolated uncultured targeted exosomes has been suggested. The effect of progenitor cell micrograft is that hair loss is treated by maintaining the micro environment by side population phenomena, niche concept, and extracellular matrix (ECM). In this experiment, a female patient in her 50s was observed for about five months from the micrograft in June until October 2023, and the experimental equipment used in this experiment was the Bio-Q developed by a local specialized technology development project. For the experiment, a biopsy of 2,5 ∅ 3 punches was performed on the back of the patient's head where there was no hair loss, and about 5cc (0.1cc each) of suspension (targeted exosomes) was immediately injected on the same day into 50 sites on her scalp where hair loss occurred, and the hair growth status was investigated afterward. Hair growth occurred at the micrograft sites, but many hairs with weak hair growth that had not yet penetrated the epidermal layer were observed growing in coils at the lower part of the epidermal layer, and the length of the coils was also found to increase at the bottom of the scalp. The number of hairs with hair growth capability increased rapidly after discovering the cause of the coil phenomenon.

Keywords : Hair loss, Microtransplant, Exosomes, Bioptic

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1. Introduction

The hair loss market is expanding rapidly due to the increase in the hair loss population domestically and abroad. New products are being developed for each medical field by subdividing into hair loss management products, devices, and medical procedures related to cell transplantation. The existing hair transplantation method is not only painful but also has serious side effects after surgery for hair loss patients, such as loss of sensation and scarring over six months. The latest non-surgical hair loss treatment involves applying a technology to extract and separate a patient's specific autologous cells and injecting a fixed amount of the isolated autologous cells to treat hair loss as the cells differentiate and proliferate. There is no pain or scarring, and because it significantly improves hair loss treatment, it is being presented as an efficient and innovative treatment method for hair loss patients. In this study, autologous hair follicles were extracted from the patient's scalp with many progenitor cells behind the ears, where there was little hair loss. Then, they were put in a cell extractor, smart chamber (Blo-Q), and separated using a spin machine. Afterward, the isolated uncultured targeted exosomes were injected into the sites requiring hair loss treatment using a syringe. In this study, a method of reinjecting autologous cells into the patient's hair loss sites using autologous micrograft, one of the non-surgical treatments, was applied. The progenitor cells from the hair loss patient's scalp behind the ears, where there is little hair loss, were punched to a size of 2.5Φ for biopsy and three biopsies were ground using a smart chamber (Bio-Q), an equipment researched and developed with local specialized technology. They were then injected into each hair loss site. The patient was observed for a total of 5 months for a quantitative evaluation by analyzing the cause of the coil phenomenon found in specific sites and analyzing hair growth in each site.

2. Theoretical Background

The progenitor cells (PCs) obtained by biopsy from the scalp behind the patient's ears were ground in Bio-Q, and 5 to 7 cc of the extracted uncultured targeted exosomes suspension was injected in a fixed amount of 0.1 cc into each hair loss site of the patient. Afterward, the hair loss sites were continuously observed for five months. Hair growth occurred in the hair loss sites, but in the case of the hair that had not yet penetrated the epidermal layer, it was observed through a digital microscope that they were growing in coils at the lower part of the epidermal layer. During the hair growth process, numerous coils appeared on the patient's scalp, and the number and length of the coils increased. Although the exact cause of the coil phenomenon has not

been identified, one of the causes is believed to be the thickening of the scalp keratin layer and the accumulation of shampoo residue due to the gentle rubbing of the scalp during hair washing after micrograft. Another cause is believed to be that when grinding the biopsy during the progenitor cell extraction process, the blade of the lower plate forcibly tore the cells, and the cells were damaged by frictional heat from the scrape on the upper plate, resulting in decreased cell activity. Therefore, after the progenitor cells (PCs) which are ground in the Bio-Q are injected into the patient's hair loss sites in the form of a suspension (targeted exosomes) and after the hair loss treatment sites are well recovered, if the hair loss treatment sites are washed with a brush using appropriate pressure and rinsed thoroughly 2-3 times or more after washed with the scalp cleaning shampoo, the occurrence of coils can be minimized. After confirming the initial occurrence of the coil phenomenon, with various measures taken, the coil phenomenon is expected to be eliminated over time. The coil phenomenon occurred after the micrograft, as shown in [Fig. 1].

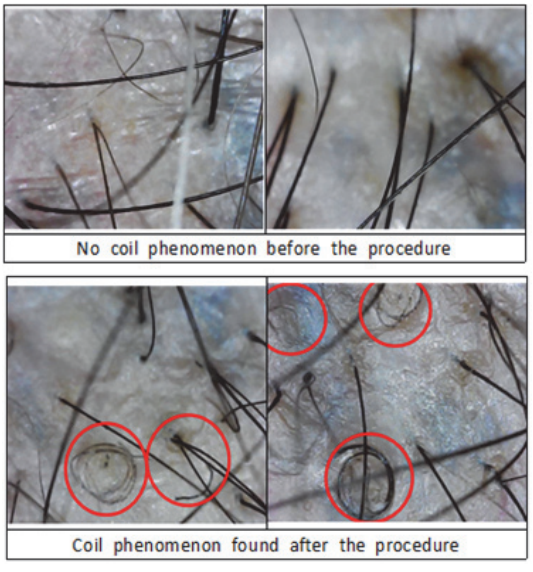


Fig. 1 Coil phenomenon before and after the hair loss treatment

Another cause of the coiling phenomenon is thought to be the strong side population phenomenon of progenitor cells (PCs), niche concept, and a decrease in activity due to signaling of the extracellular matrix (ECM), that is, those growing hairs are too thin and their activity decreases. This appears to be because they cannot penetrate the epidermal layer, and the phenomenon occurs with hairs with weak penetration during the process of hair growth of a large amount of other hairs. Moreover, numerous papers have presented that progenitor cells (PCs) survive only in populations with fixed morphological features. The main morphological features are the size and expression of stem cell markers. Only by carefully preserving the side population phenomenon, niche concept, and extracellular

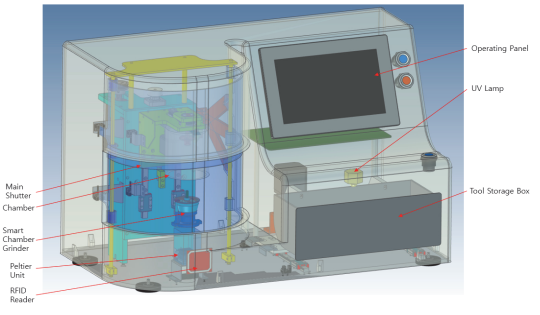


Fig. 2 Smart chamber separation machine

matrix (ECM) of progenitor cells extracted using smart machines [Fig. 2] and Bio-Q [Fig. 3], it will be possible to maintain a physiological microenvironment that is favorable for cell survival. Therefore, this study will provide appropriate physiological factors for progenitor cells by designing the microenvironment (niche) to help cells survive by installing a Peltier device to prevent frictional heat caused by grinding at the bottom of the smart chamber.

Meanwhile, the physiological factors of progenitor cells regulate various vital phenomena such as cell proliferation, differentiation, and death.[1] For example, physiological factors such as hormones, growth factors, and cytokines each regulate cell proliferation, differentiation, and immune responses.[2] Some physiological factors also show anti-aging effects, such as suppressing cellular aging.[3] Physiological factors can also affect cell shape and motility.[4] Endothelial progenitor cells or stem cells may be involved in developing peripheral blood vessels.[5] A large number of exosomes in the hair follicle progenitor cells can be extracted using the smart chamber separation machine, Bio-Q. Hair follicles are one of the adult stem cells, and hair follicle stem cells and progenitor cells play an important role in hair follicle regeneration. If hair follicle stem cells and progenitor cells decrease, hair growth may decrease, and hair loss may occur.[6] Mesenchymal and epithelial cells are necessary for hair follicle regeneration, which can be derived from hair follicle stem cells and progenitor cells.[7] Hair follicles go through anagen(growing phase), catagen(transition phase), and telogen(resting phase) stages, and activation of stem cells and progenitor cells is important during this process.[8] The Regenera Activa technology developed by the Italian company HBW [Fig. 4], which has recently been used in many countries, uses the patient's own hair follicle progenitor cells to treat hair loss and thinning hair.[9]

Uncultured targeted exosomes extracted from progenitor cells (PCs) are small vesicles that play an essential role in cell-to-cell

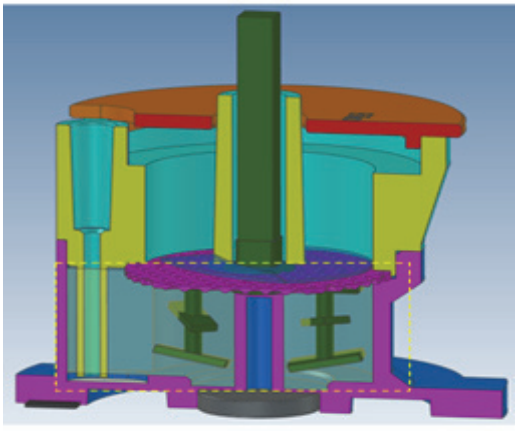


Fig. 3 Bio-Q

Fig. 4 HBW company`s regenera machine and con

communication, and in this study, they are thought to affect many physiological processes, including tissue regeneration and repair by transferring from one cell to another, including proteins, RNA, and other molecules. On the other hand, Wnt signaling is well-known as a protein network that regulates cell-to-cell interactions during embryonic development and is expected to play an important role in controlling cell growth, migration, and differentiation. The progenitor cells that are ground in the smart chamber separation machine, Bio-Q, are widely used as a treatment that utilizes these biological processes to stimulate the scalp and promote hair growth. By utilizing natural signaling pathways and growth factors such as Wnt and BMP, the hair follicle environment can be improved, including the density and thickness of the hair.

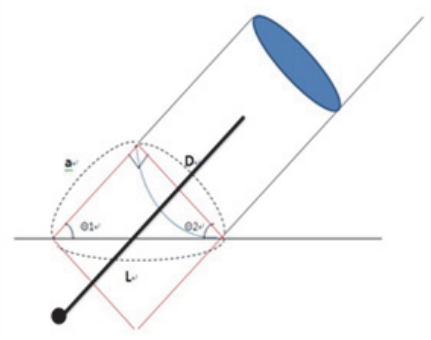
Hair regeneration-promoting Wnt activator induces activation and proliferation of hair follicle stem cells to promote hair regeneration,[10] fat cell differentiation inhibiting Wnt signal pathway reduces factors that interfere with hair growth,[11] bone formation-promoting Wnt activator aids bone regeneration by increasing bone cell survival. Therefore, the Wnt activator can be effectively used not only for hair regeneration but also for various tissue regeneration.[12] Moreover, the regenerative capacity of stem cells and progenitor cells increased through activation of the Wnt/β-catenin signaling pathway.[13] Due to this, stem cell therapy is expected to be commercialized for the treatment of spinal cord injury for the first time in the world.[14] A phase 1/2a clinical trial targeting patients with knee osteoarthritis is in progress for treatment with autologous adipose-derived chondroprogenitor cells.[15] Also, a treatment for endothelial progenitor cells is being developed, and safety and economic feasibility are secured using natural substances.[16] Through various research institutes domestically and abroad, technology has been developed to differentiate human pluripotent stem cells into hepatocytes in large quantities, which is expected to make it possible to manufacture liver tissue regeneration treatments.[17] Meanwhile, another technology has been developed to control differentiation into specific cell and tissue series, which will make it possible to manufacture customized cell therapies for the treatment of various diseases.[18] In efforts to overcome hair loss, various treatments and procedures have been proposed worldwide, but the reality is that no method that satisfies all types of hair loss patients has been found to date.

3. Experimental Method

3.1 Biopsy Sample

For this study, the hair loss condition of a female patient in her 50s was observed with the naked eye prior to micrografting. From

its results, the hair loss patient showed a typical female pattern of hair loss as her hair rapidly thinned after giving birth. For the progenitor extraction method and equipment used for patient-specific hair loss treatment in the experiment, progenitor cells can also be extracted using "Zeus."[19] According to John P. Cole, scars smaller than 1.5 mm left after incisions are generally difficult to see with the naked eye. In fact, when hair follicles were collected by units with punches of 1.25 mm and 1.00 mm in diameter, the results after recovery were the same. Even if a punch of the same size is used, the size and depth of the incision may vary depending on the punching angle. The punch angle is adjusted to the angle at which the hair grows out of the scalp, and how it changes is shown in [Fig. 5], and the formula between the variables is as follows.[20]



L: Length. When the punch is inserted, the skin surface is cut into an oval shape and the length of the long axis is marked as L.
D: Diameter. The diameter of the punch
a: Depth. Insert the punch according to the angle of hair growth

Fig. 5 The angle of the punch dapends on the angle of hair growth

Using a punch that is too small compared to the size of the hair follicle unit increases the possibility of hair follicle damage, and using a punch that is too big (diameter of 1.5 mm or more) can result in different scar sizes. Hence, it is important to select a punch of the appropriate size.[20] The depth of collecting hair follicles (progenitors) for non-incisional hair transplantation or non-surgical hair loss treatment basically aims to punch to the depth just below the sebaceous glands. However, in actual surgeries, it may be performed differently by different surgeons depending on the characteristics of the hair follicle.[21] In this experiment, a female hair loss patient in her 50s was observed for five months after an

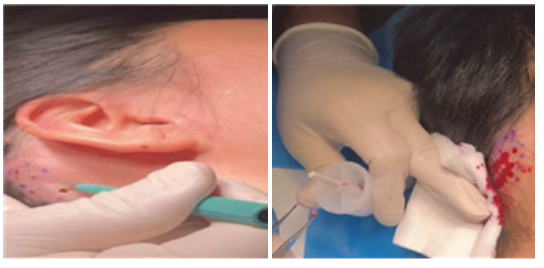


Fig. 6 Anesthesia and punching, Biopsy

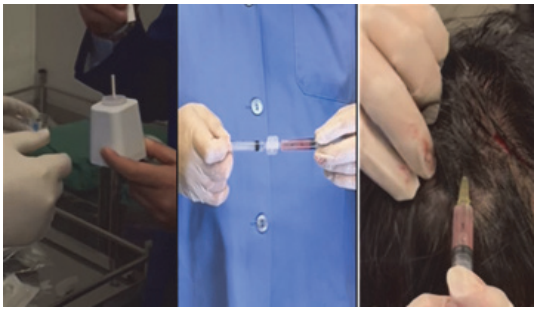


Fig. 7 Grinding, Agitation, Injection

autologous micrograft in June 2023, and in the back of the patient's head, where there is no hair loss, biopsy [Fig. 6], grinding and agitation [Fig. 7] processes were performed, and about five ccs of uncultured targeted exosome suspension was prepared, and immediately on the same day, as shown in [Fig. 7], a fixed amount of 0.1 cc was injected into approximately 50 sites where hair loss was occurring. The condition of the scalp was imaged one month after the procedure, and data on hair growth in hair loss sites of the scalp were collected and observed.

3.2 Selection of Procedure Sites

First, the sites where the biopsy and micrograft will be performed were selected before the procedure, and a fixed amount of 0.1 cc was injected into the patient's scalp.

Table 1. Camera Specification

Digital Microscope · SPECIFICATION	
IMAGE SENSOR	CMOS
IMAGE RESOLUTION	Up to 640*480, 1920*1440
FOCUS RANGE	15mm-40mm
AVAILABLE IMAGE FORMAT	BMP/JPG
ADJUSTABLE ILLUMINATION	8 Built-in LED Diodes
COMPATIBLE OS	Windows 7, Windows 10/Mac 10.13 and above
MOBILE PHONE COMPATIBLE OS	Android
DIMENSION	14.4cm x 10cm x 5cm

Progress is monitored once a month for a total of four times using equipment of the same specification, such as a digital microscope [Table 1]. From a total of five spots in sections A-E [Fig. 8], the images were taken and compared to be analyzed.

The images were taken at five spots in the A-E section for four months, and the imaging distance from the lens to the epidermis layer was 15 mm, the lens focal length was 15mm, and the fixed focus imaging area was W=2.88/15x2.88=2.88 mm^2 and vertical H =2.1/15x15=2.1 mm. The Lens calculator was applied to measure the distance between the camera lens, angle of view, and focal length, and the camera used for imaging was a digital microscope [Fig. 9]. Here, when applying formula 1 below and applying the image sensor format type and size specifications in [Table 1] based on 1/5 CCD, it was W: 2.88 and H: 2.16. Therefore, when formula 2 is applied to 1 imaged pixel of 2.88 mmx2.16 mm, the scalp imaging area per 1 imaged sector becomes 6.2208 mm^2 .

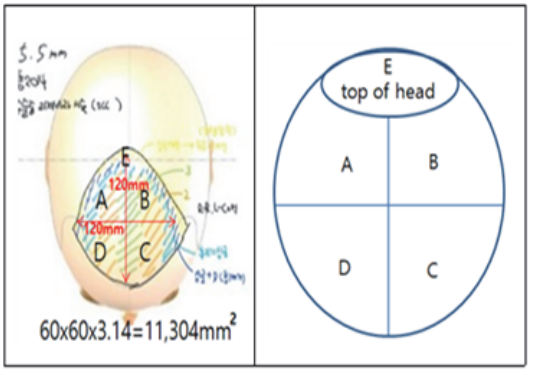
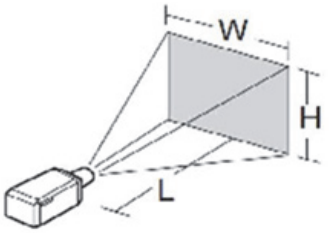


Fig. 8 5 video acquisition point of A-E section



■For $\frac{1}{3}$ - type lens .....formula(1)

$$W=\frac{4.8}{f}\times L \qquad H=\frac{3.6}{f}\times L$$

■For $\frac{1}{4}$ - type lens

$$W=\frac{3.6}{f}\times L \qquad H=\frac{2.7}{f}\times L$$

■For $\frac{1}{5}$ - type lens

$$W=\frac{2.88}{f}\times L \qquad H=\frac{2.16}{f}\times L$$

$$W=\frac{2.88}{f(15)}\times L(15)=2.88mm^2$$

$$H=\frac{2.16}{f(15)}\times L(15)=2.16mm^2$$

.....formula(2)

4. Experiment Results and Discussion

In this experiment, images acquired monthly for four months at five spots (A-E) for a female hair loss patient are shown in [Fig. 10]. As show nin the area W2.88x

Fig. 9 Digital microscope used for scalp imaging

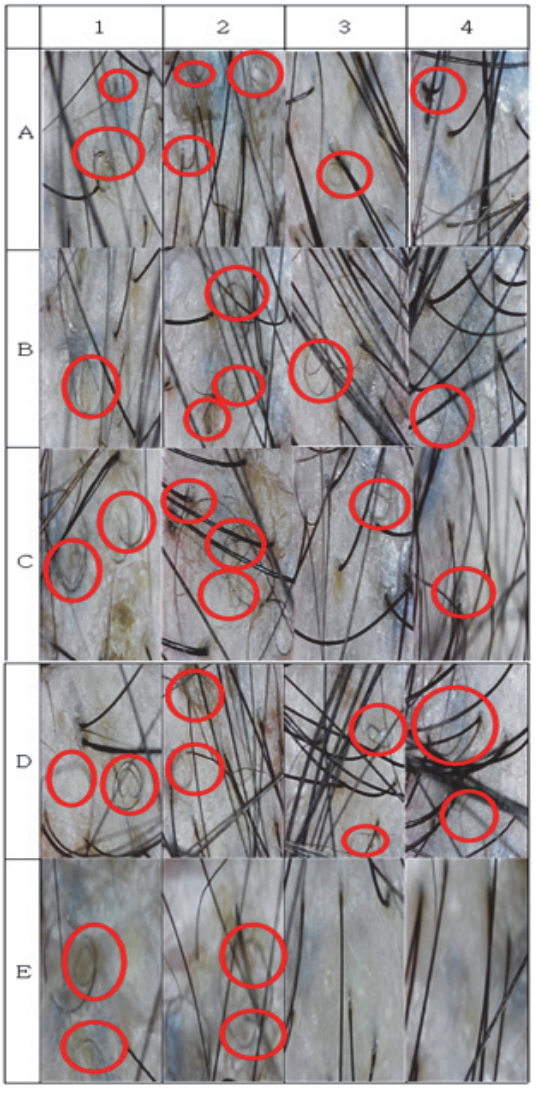


Fig. 10 Images acquired from 5 spots in sections A-E for 4 months

H2.16mm=6.2208 of the 1 imaged sector, a large number of coils were found after the first micrograft. However, after two months of image acquisition, after washing with a brush and scalp shampoo and rinsing it thoroughly, the patient's hair growth increased exponentially, as shown in [Fig. 10] and [Table 2].

The average number of coils per 6.2208 mm^2 sector of the image obtained was 1.8 in month 1 after the micrograft, and the number of hairs after the micrograft was 3.2. In month 2, the average number of coils was 2.6, and the number of hairs was 6.2. In month 3, the average number of coils was 1, and the number of hairs was 7.8. In month 4, the number of coils was 1, and the number of hairs was 8. The data and analysis table figures are shown in [Table 2]. The average number of coils in month 2 increased slightly compared to month 1, but as the number of months increased, the number of coils decreased while the number of hairs increased. The result was confirmed by the patient herself, checking the condition of her scalp with her naked eye at the time of starting the analysis. The reduction of the number of coils and the increase in the amount of hairs were confirmed after the patient used a brush with appropriate strength, washed her hair with shampoo, and rinsed it sufficiently 3-4 times or more.

Table 2. Analysis table for image data before and after the procedure month 1 to month 4

	Before the procedure		Month 1		Month 2		Month 3		Month 4		Sector average	
	Coils	Hairs	Coils	Hairs	Coils	Hairs	Coils	Hairs	Coils	Hairs	Coils	Hairs
A	0	3	2	4	3	7	1	9	1	9	1.75	7.25
B	0	3	1	4	3	8	1	8	1	8	1.5	7
C	0	3	2	3	3	6	1	8	1	8	1.75	6.25
D	0	2	2	3	2	8	2	10	2	10	2	7.75
E	0	2	2	2	2	2	0	4	0	6	1	3.5
Mnthy Av.	0	2.6	1.8	3.2	2.6	6.2	1	7.8	1	8.2	1.6	7.9
Total	0		4,724.54	3,270.6	5,814.82	4,724.54	11,266.21	1,817.13	14,173.61	1,817.13	14,537.04	※Rounding off to 2 decimal places

As a result of this study, when using a female hair loss patient's own progenitor cells, the amount of progenitor cell collection may be limited, and in particular, it is often difficult to secure a sufficient amount of progenitor cells required for cell therapy.[22] Due to various types of hair loss, the survival rate and function of transplanted progenitor cells are limited in the damaged tissue environment, so the treatment effect may not be sufficient, and the cell differentiation may be limited, so it may be difficult to expect the desired amount of hair growth.[23] There may be a risk of side effects such as transplant rejection and coiling phenomenon during progenitor cell therapy. Therefore, it is crucial to develop cell extraction technology and patient-tailored treatments through non-

contact grinding methods rather than using particle plates or scrapes for grinding biopsies. There is also a risk of cancer.[24] Oligodendrocyte progenitor cells (OPCs) play an important role in supporting sensory information and learning experiences associated with neural circuit changes.[25] Adult stem cells are distributed in almost all tissues in tissue regeneration and homeostasis maintenance.[26] Hematopoietic stem cells differentiate into blood cells and vascular endothelial cells. In this experiment.[27] assuming the area of each unit sector (σ) of 'A,' 'B,' 'C,' 'D,' and 'E' is 6.2208 mm^2 , and the area of the entire hair loss treatment (injection) site (Σ) is 120 mm by 120 mm, 60 mm x 60 mm x 3,14 equals 11.304 mm^2 , according to the area of circle . The total number of hair loss treatment (injection) area sectors (ε) is the total hair loss treatment (injection) area (Σ)/unit sector area (σ)$=\frac{\Sigma}{\sigma}=\frac{11{,}304mm^2}{6.2208mm^2}=$ 1,817.1296(ε). σ: Unit sector area, Σ: Total hair loss treatment (injection) site area, ε: Total number of hair loss treatment (injection) site sectors.

[Fig. 11] is the process of hair number change according to the number of months per 5 unit sector area (σ) of A to E after the procedure. [Fig. 12] is the process of coil number change according to the number of months per 5 unit sector area (σ) of A to E after the procedure. [Fig. 13] presents the process of a monthly number of coil and hair changes in the entire area of the hair loss treatment (injection) sites (Σ) after the procedure, and the derived value is the product of multiplying the monthly average number of coils and hairs in the unit sector area (σ) and the number of sectors (ε) in the entire hair loss treatment (injection) area.

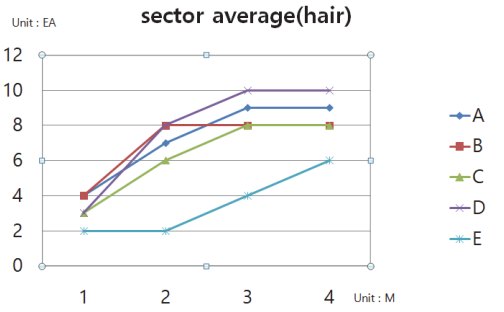


Fig. 11 No. of hair change by unit sector area (σ)

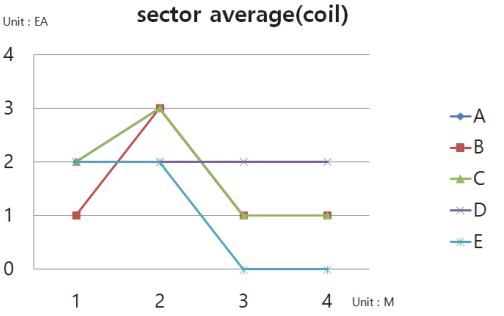


Fig. 12 No. of coil change by unit sector area (σ)

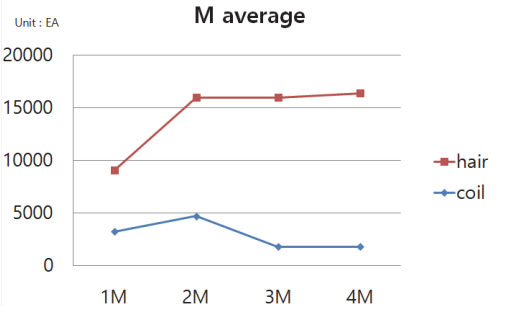


Fig. 13 No. of monthly coil and hair changes in the entire hair loss area (Σ)

The overall number of hairs increased as the number of months increased, as shown in [Fig. 11] and [Fig. 13], and the number of coils increased, as shown in [Fig. 12] and [Fig. 13], there was a slight increase overall between months 1 and 2, but it generally decreased in months 3 and 4. In conclusion, it is believed that hair growth is improved by the targeted exosome injection procedure of 5cc, which involves thoroughly rinsing the hair after washing it with shampoo and physical friction after the autologous micrograft.

5. Conclusion

In this study, to accurately determine the number of coils and hairs found in the area imaged by the equation that counts lens focal distance [Table 3] and the pixels, the patient's scalp was divided into five sectors, and the images were acquired. The number of coils and total hairs for each sector were checked, and the number of hairs generated each month was counted.

The position and angle of monthly imaging were not always the same, so in the experiment, the patient's scalp was divided into five spots. To minimize numerical changes, the measurement was performed by one person, and the images were acquired at the same time and location as much as possible in each month to obtain the average value.

However, the image adjustment and imaging area of the entire images were not quite efficient in terms of accuracy, so as a correction process to find the actual spots of

Table 3. image Sensor Format Type size

			4:3 Aspect Ratio		3:2 Aspect Ratio	
Sensor Format Type	Real Calc Diag (mm)	Format Type Diag (mm)	Width (mm)	Height (mm)	Width (mm)	Height (mm)
35mm Full Frame	N/A	43.30	34.64	25.98	36.03	24.02
APS-C	N/A	30.10	24.08	18.06	25.04	16.07
4/3"	33.87	21.33	17.07	12.80	17.75	11.83
1.1"	27.94	17.60	14.08	10.56	14.64	9.76
1"	25.40	16.00	12.80	9.60	12.31	8.88
1/2.0"	12.70	8.00	6.40	4.80	6.00	4.44
1/3.0"	8.47	6.00	4.80	3.60	4.99	3.33
1/4.0"	6.35	4.50	3.60	2.70	3.74	2.50
1/5.0"	5.08	3.60	2.88	2.16	3.00	2.00
1/6.0"	4.23	30..	2.40	1.80	2.50	1.66
1/7.5"	3.39	2.40	1.92	1.44	2.00	1.33
1/9.0"	2.82	2.00	1.60	1.20	1.66	1.11

16:10 Aspect Ratio		16:9 Aspect Ratio	
Width (mm)	Height (mm)	Width (mm)	Height (mm)
36,72	22,95	37,74	21,23
25,52	15,95	26,23	14,76
18,09	11,30	18,59	10,46
14,92	9,33	15,34	8,63
13,57	8,48	13,95	7,84
6,78	4,24	6,97	3,92
5,09	3,18	5,12	2,94
3,82	2,39	3,92	2,21
3,05	1,91	3,14	1,76
2,54	1,59	2,61	1,47
2,04	1,27	2,09	1,18
1,69	1,06	1,74	0,98

each image, previous images were compared with the images taken in that month, and only images showing similar phenomena in predefined areas ('A,' 'B,' 'C,' 'D,' and 'E') were accepted.

As a result of this study, first, in the case of a middle-aged woman who participated as a subject, her hair loss started after giving birth, and the hair thickness naturally became thinner, making it difficult to secure a sufficient amount of progenitor cells.

Second, if healthy progenitor cells(targeted exosomes) were not transplanted, the hair growth weakened, resulting in a prominent coil phenomenon as the hair failed to penetrate the epidermis layer.

Third, in the case of the female subject's hair loss, the incidence rate of coils was high due to the difficulty in obtaining sufficient progenitor cells, the build-up of keratin in the epidermal layer of the scalp, or the failure to rinse thoroughly after washing hair.

Fourth, the survival rate and hair activity after autologous micrograft using progenitor cells ldecrease due to cell damage caused by physical friction, frictional heat, and long-term external exposure from grinding. As a result, it seems that the hair cannot penetrate the epidermal layer, so a coil phenomenon occurs in the subcutaneous layer.

In this study, to examine the hair condition of a hair loss patient, a camera equipped with high-efficiency LED light was used to recognize the hair in the image output from a digital microscope and to make the blurred images clearer. Further study of the non-contact high-pressure waterjet grinding method is necessary to solve various issues caused by the contact grinding method in this study.

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