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Gold thread implantation promotes hair growth in human and mice

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Thread-embedding therapy has been widely applied for cosmetic purposes such as wrinkle reduction and skin tightening. Particularly, gold thread was reported to support connective tissue regeneration, but, its role in hair biology remains largely unknown due to lack of investigation. When we implanted gold thread and Happy Lift™ in human patient for facial lifting, we unexpectedly found an increase of hair re-growth in spite of no use of hair growth medications. When embedded into the depilated dorsal skin of mice, gold thread or polyglycolic acid (PGA) thread, similarly to 5% minoxidil, significantly increased the number of hair follicles on day 14 after implantation. And, hair re-growth promotion in the gold thread-implanted mice were significantly higher than that in PGA thread group on day 11 after depilation. In particular, the skin tissue of gold thread-implanted mice showed stronger PCNA staining and higher collagen density compared with control mice. These results indicate that gold thread implantation can be an effective way to promote hair re-growth although further confirmatory study is needed for more information on therapeutic mechanisms and long-term safety.

Keywords: Gold thread, hair loss, hair growth

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The number of aged population rapidly grows in modern societies and thereby the average age of socially active groups tends to become higher. Concurrently, an increasing number of people become to have interest in anti-aging therapies due to psychological and aesthetical reasons, and visit plastic surgeons for consultation. Particularly, the aging of the mid-facial region occurs with descent of soft tissue and loss of contour of neck [1], and thread-embedding therapy has been used as a type of dermal needle therapy for wrinkle reduction and skin tightening in such region possibly through the regeneration of connective tissue [2,3]. Besides its use in plastic surgery, thread therapy has also been applied to various chronic diseases such as chronic gastritis, bronchial asthma, and allergic rhinitis in East Asia [4-7]. Recently, Shin *et al.* [8] reported that thread therapy promoted hair growth through the regulation of anagen-associated growth factors and activation of the proliferation of hair follicles

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following skin depilation, suggesting its potential in treating hair loss.

Hair loss or hair thinning is a common hair disorder associated with the dysregulation of the hair growth cycle [9-12], resulting from aging, alteration of hormone secretion, nutritional imbalances, social stress, and genetic factors [12,13]. Although it is not a life-threatening problem, hair loss significantly decreases patients' psychological well-being and quality of life by causing formation of inappropriate personal relations with other people. Although several drugs such as finasteride and minoxidil have been approved by the US Food and Drug Administration (FDA) for promoting hair re-growth and protecting hair loss [14], their efficacies are transient since hair loss may resume after discontinuation of use [15-19]. In this study, we tested whether dermal implantation of gold thread facilitated hair re-growth in human and mice. Our findings suggest that gold thread is a safe and effective hair growth stimulant.

Materials and Methods

Determination of hair growth-promoting activity in human patient

One female patient aged 38 years performed a facial lifting using thread for facial rejuvenation and participated in this study. This patient confirmed she did not use hair growth medications or hair enhancement products. The implant materials were an nonabsorbable, monofilament, suspension thread Happy Lift™ (Promoitalia International S.R.L, Naples, Italy) and 0.1 mm diameter gold thread (Lorca Marin, Murcia, Spain) braided with a strand of absorbable polyglycolic acid (PGA). The procedure was performed with one patient under local anesthesia with 2% lidocaine and diluted epinephrine (1:100,000). Gold threads and Happy Lift™ were inserted in the sub-dermal layer of the skin (Figure 1A). The hair re-growth was assessed using post-implantation photographs taken after 24 months after the operation. The patient who participated in this study accepted and signed the necessary informed consent. And, this protocol was approved by the e-IRB system of Korea National Institute for Bioethics Policy site as an exempt protocol.

Determination of hair growth-promoting activity in mice

C57BL/6J mice (Orient Bio, Seongnam, Korea) were acclimatized to laboratory conditions for 1 week with their health status carefully monitored before the initiation of the study. Animals were housed in a humidity (40-60%) and a temperature (22±2°C) controlled AAALAC International (#00169) accredited facilities with a 12-hour light and 12-hour dark cycle, and a commercial rodent diet (LabDiet 5002 Certified Rodent Diet, PMI Nutrition International, St. Louis, MO, USA) and tap water were supplied *ad libitum*. 7-week-old C57BL/6J mice were randomly allocated into the following four groups (n=19 per group). Group 1 was normal saline-

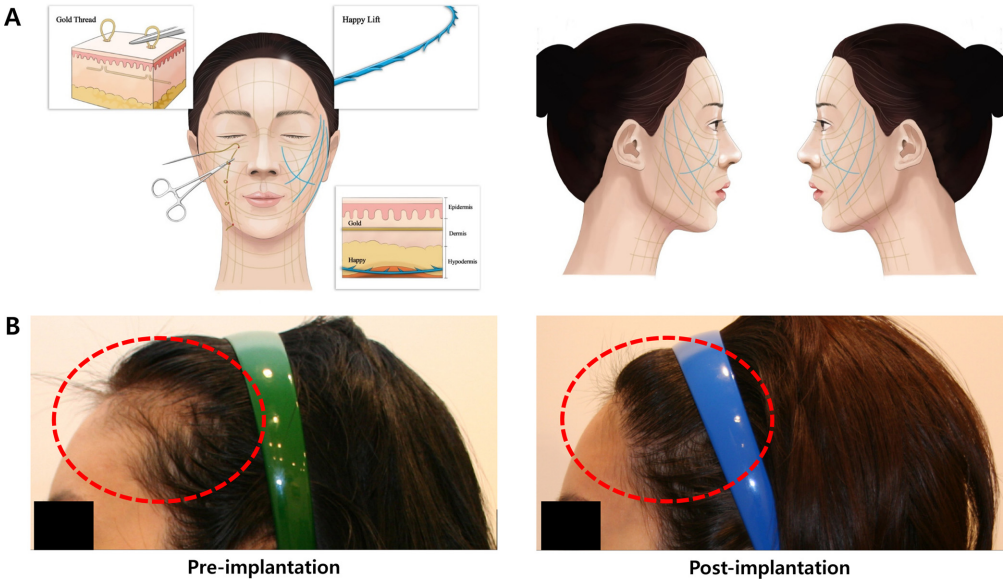

Figure 1. Comparison of hair growth in human. (A) Schematic procedure line of gold thread and Happy Lift™ implantation in human. (B) Representative photographs of human patient with or without gold thread and Happy Lift™ implantation.

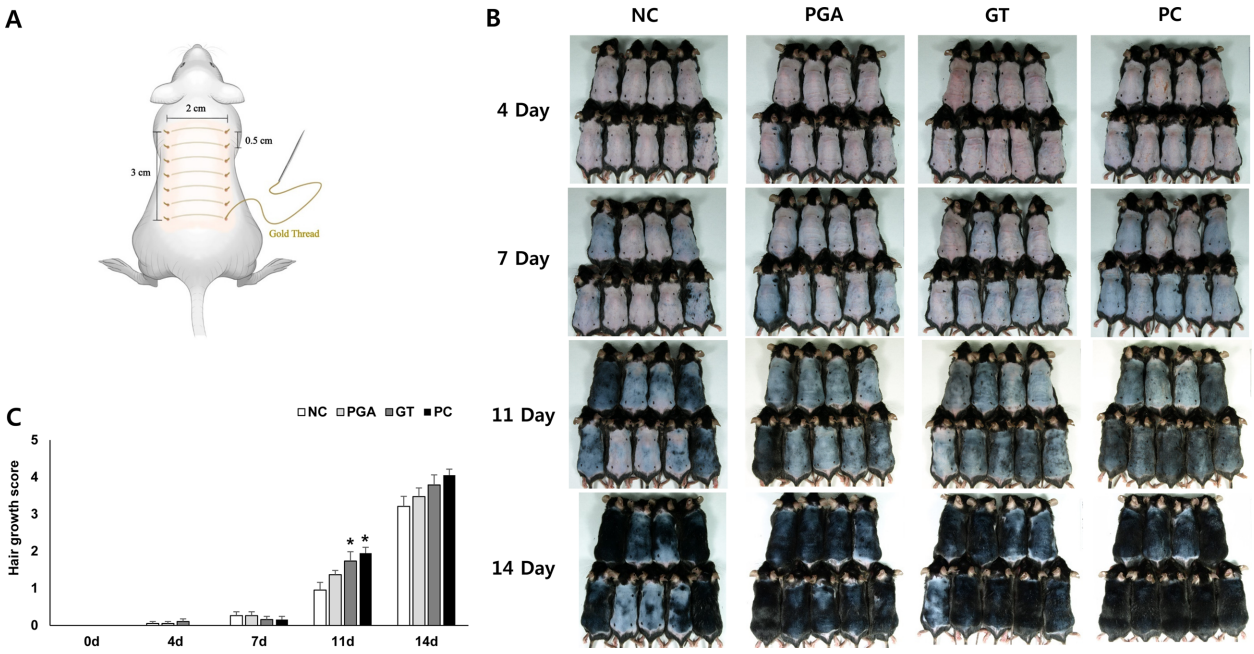

Figure 2. Comparison of hair growth in mice after depilation. (A) Schematic procedure line of gold thread implantation in mice. (B) Photometric comparison of hair growth on day 4, 7, 11, and 14 after depilation. (C) Hair growth scores using a scoring index (0=no growth, 1=up to 20% growth, 2=20-40%, 3=40-60%, 4=60-80%, and 5=80-100%). Values in the graph are expressed as means±SEM (n=19/group). *; Significantly different compared to normal saline-treated negative control group (P<0.05). NC, negative control; PGA, polyglycolic acid thread; GT, gold thread; PC, positive control.

applied negative control, and Group 2 was 5% minoxidil (Hyundai Pharm, Seoul, Korea)-treated positive control. Group 3 and 4 were absorbable PGA thread (Ailee, Busan, Korea)- and gold thread-embedded experimental groups, respectively. For anagen synchronization, the dorsal hair of mice in the telogen phase was artificially shaved before the beginning of the experiment using an electric clipper for animals [20]. One day after removal of the dorsal hair, the mice in Group 1 and 2 was topically treated with normal saline or 5% minoxidil using micro pipettes (200 μL per application), while the mice in Group 3 and 4 were implanted with seven PGA or gold threads about 2 cm in length at intervals of about 0.5 cm in the dorsal skin (6 cm^2). The dorsal skin lesions of the mice were photographed with a digital camera under anesthesia and the hair growth was rated on day 0, 4, 7, 11, and 14 following depilation by assigning a hair growth score ranging from 1 to 5 (0=no growth, 1=up to 20% growth, 2=20-39% growth, 3=40-59% growth, 4=60-79% growth and 5=80-100% growth) according to the method of analysis as described by Kwon *et al.* [12]. At scheduled termination, the mice were sacrificed and dorsal skin tissue was excised. All procedures on animals were performed in accordance with Guide for the Care and Use of Laboratory Animals 8th edition [21] and the study protocol was approved by the Institutional Animal Care and Use Committee of the Biomedical Research Institute at the Seoul National University Hospital.

Histological observation of hair follicles and collagen in dermis

Individual skin samples of mice were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E) for histopathological evaluation. The number of hair follicles in the skin samples was counted under a light microscope (IX61, Olympus, Tokyo, Japan). Also, Masson's trichrome staining was performed to evaluate the amount of collagen in the dermis. For immunohistochemistry, slides were incubated with anti-Ki67 antibody (Abcam, Cambridge, MA, USA) or anti-proliferating cell nuclear antigen (PCNA) antibody (Abcam) for 32 min at 37°C, and a secondary antibody (UltraMap anti-RB HRP, Ventana Medical Systems, Inc., Tucson, AZ, USA) for 20 min at 37°C.

Statistical analysis

All data are expressed as mean±SEM. Statistical significance was tested using one-way ANOVA followed

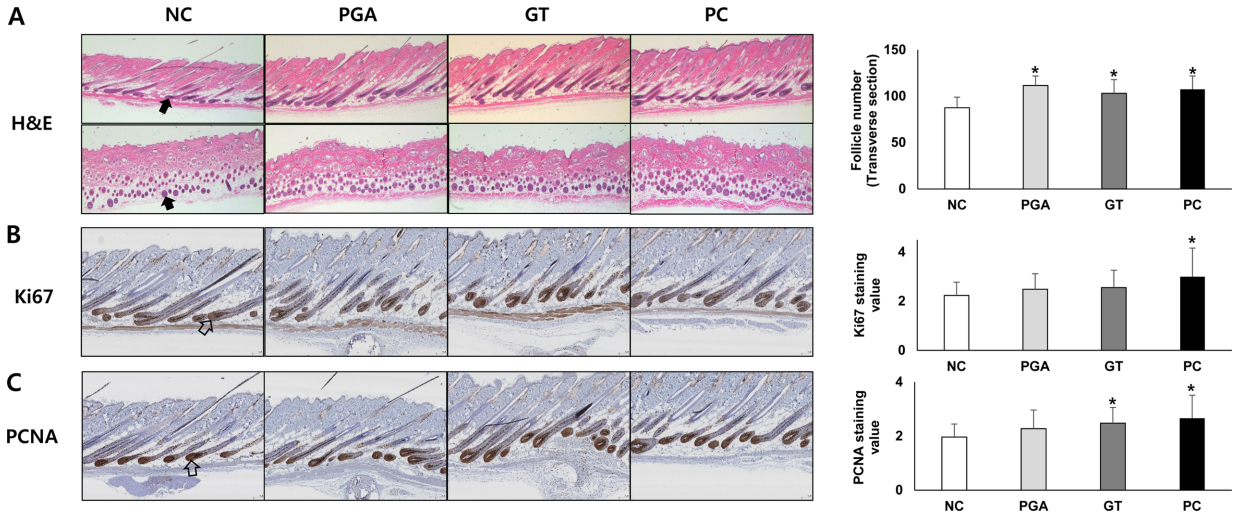

Figure 3. Effect of gold thread on hair follicles in mice. (A) Photomicrographs of H&E stained sections of dorsal skin. Upper panels show longitudinal sections and lower panels show transverse sections. The number of hair follicles (closed arrows) in transverse sections. (B, C) Histologic values of Ki67 (B) and PCNA (C) positivity in dorsal skin. Strong reactions of Ki67 and PCNA were observed in hair bulb (open arrows). Values in the graph expressed as means±SD. *; Significantly different compared to normal saline-treated negative control group (P<0.05). NC, negative control; PGA, polyglycolic acid thread; GT, gold thread; PC, positive control.

by *post hoc* Dunnett's multiple comparison test in a SPSS software version 19 (SPSS Inc., Chicago, IL, USA). A P value of <0.05 was considered to be statistically significant.

Results

Dermal implantation of gold thread promoted hair re-growth after depilation

Figure 1B presents gross images of hair growth. When we implanted gold thread and Happy Lift™ in human patient for facial lifting, we unexpectedly found significant improvements in hair re-growth. Based on these results, we further assessed the potential effect of gold thread on hair growth in animal study.

Hair growth cycle of a C57BL/6 mouse has been known to have a time-synchronized following depilation [20]. Therefore, we shaved the dorsal skin of mice and assessed the effect of gold threads on hair growth by implanting them under the dorsal skin (Figure 2A) and observing the degree of hair re-growth. Calculation of hair growth scores (see Materials and methods for scoring criteria) on day 11 after depilation resulted in showed 0.95, 1.95, 1.37, and 1.74 for normal saline, 5% minoxidil, PGA thread and gold thread-treated groups (Figure 2B, 2C). The scores of 5% minoxidil and gold thread groups were significantly higher than the normal saline group (P<0.05), suggesting hair growth promoting activity, while no differences were observed between the normal saline and PGA thread groups. Although statistical significance was not observed, the higher hair growth scores of the 5% minoxidil-treated and gold thread-treated groups than the normal saline-treated group were maintained to 14 days after depilation. These results suggest that insertion of gold thread may increase hair growth by positively influencing the hair cycle in C57BL6/J mice.

Histological observation of hair follicles in the C57BL/6 mouse model

Hair follicles in H&E-stained skin tissues from the shaved dorsal skin of mice were observed using optical microscopy and evaluated on day 14 after depilation (Figure 3A). The dorsal skin of the gold thread-treated mice was stained with H&E and histopathologically assessed using an optical microscope. Data acquired on day 14 indicated that the number of hair follicles significantly increased in the groups treated with gold thread (103.09±3.39), PGA thread (111.56±2.35), and 5% minoxidil (107.44±3.29) than in the normal saline-treated group (87.51±2.63). In particular, PCNA expression was significantly increased in gold thread group although 5% minoxidil group increased both Ki67 and PCNA expressions, standard markers of active cellular proliferation (Figure 3B, 3C) [22]. These results, together with our findings on hair growth score, suggest that gold thread

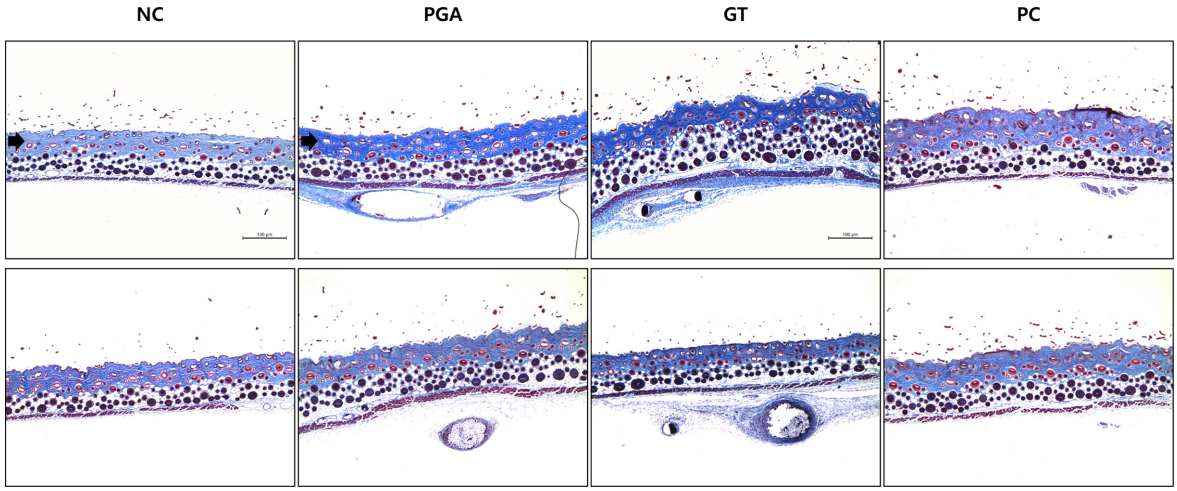

Figure 4. Effect of gold thread on collagen in dermis. Photomicrographs of Masson's trichrome stained sections (arrows) in dermis of dorsal skin. NC, negative control; PGA, polyglycolic acid thread; GT, gold thread; PC, positive control.

implantation induces hair growth possibly through promoting conversion from early telogen to anagen in hair follicles of mice.

The amounts of collagen (blue color) in dermis determined by Masson's trichrome staining were markedly increased in the gold thread, PGA thread, and 5% minoxidil-treated groups when compared with the normal saline-treated group on day 14 (Figure 4). In particular, the skin tissue of gold thread-implanted mice showed the highest density of blue-colored collagen deposition.

Discussion

Various methods are currently available for skin rejuvenation and wrinkle reduction from surgical approaches such as facelifts to nonsurgical procedures including botulinum toxin, laser, and fillers [23-25]. Recently, thread therapy, the subcutaneous insertion of surgical thread, has received great attention especially in East Asia for lifting facial tissues through the improvement of connective-tissue regeneration, collagen synthesis, skin elasticity [2,3]. The implantation of gold thread, which was first introduced by Dr. Caux in France, has been also known to improve the skin structure and elasticity by increasing collagen production and thus slow the aging process such as wrinkle formation [26,27]. Interestingly, Shin *et al*. [8] have reported that thread therapy using Polydioxanone medical thread enhanced the growth of hair follicles through the regulation of the expressions of fibroblast growth factor (FGF)-7 and FGF-5, but its effect on hair growth has not been characterized.

In the present study, gold thread implanted in human was accidentally found to be effective in promoting hair re-growth. When we assessed the effect of gold thread implantation in induced telogenic C57BL/6 mice, a well-known research model for evaluation of hair growth [20,28,29], we also found that its insertion in the depilated dorsal skin significantly accelerated hair re-growth compared with the normal saline-treated negative control group. Moreover, horizontal sectioning from H&E-stained skin tissues collected at 14 days after depilation revealed that the gold thread implantation significantly increased the number of hair follicles. Along with a clear value of stronger PCNA staining in gold thread group, H&E staining result indicates that it could promote hair growth and prevent hair loss through the significant role in hair follicle development. Mouse hair follicle cycling is known to follow regression (catagen), resting (telogen), shedding (exogen) and then regrowth (anagen), which takes about 3 weeks for completion of a cycle [30] and mouse hair follicle generally undergoes anagen phase (day 9 after depilation), catagen phase (day 17), and telogen phase (around day 20) after depilation. As hair follicle growth is directly associated with hair growth [20,31-34], our findings on the increased number of hair follicles in human and the mouse model strongly suggest that gold thread promotes hair growth.

In addition to the androgenetic theory on the etiology and pathogenesis of male pattern baldness, Ustuner [35]

demonstrated that the pressure induced by the weight of the scalp can be another cause of baldness. Since the soft tissues around the hair follicles play an important role in buffering pressure between the scalp skin and the cranial bones, the pressure on the hair follicles increases when reduction of facial soft tissues and subsequent loss of elasticity occur with aging [35]. Previously, Shin *et al.* [27] have found that gold thread implantation can promote angiogenesis, as there are rich of blood vessels over the region of gold thread implantation in comparison to other regions and we demonstrated that gold thread implantation significantly increased the amount of collagen in the induced telogenic mouse model (Figure 4) in the present study. These findings support Ustuner's theory [35], at least partially, on the etiology of baldness, proposing gold thread implantation as a potential therapy for hair loss by increasing collagen production and therefore strengthening the soft tissue surrounding hair follicles to create a stable environment for hair growth.

Although two FDA-approved drugs, finasteride and minoxidil, have been commercialized for treatment of people with hair loss, their application has been limited because of their numerous adverse effects [36], necessitating the development of an alternative option to safely treat hair loss. Our results in this study show the marked hair growth-promoting effect of gold thread implantation in both human and mice. These findings, although larger scale studies are necessary for elucidation of the therapeutic mechanisms and further validation of effectiveness and long-term safety in humans, suggest that gold thread implantation can be a potential alternative therapeutic for hair loss.

Conflict of interests The authors declare that there is no financial conflict of interests to publish these results.

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