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#### **CASE REPORT**

### Effectiveness of Microneedling Using Long Needles for the Treatment of Non-inflammatory Alopecia in Dogs

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#### ABSTRACT

Non-inflammatory alopecia includes endocrine diseases and alopecia X. Microneedling (MN) promotes hair regrowth in cases with alopecia X. However, its effects on other non-inflammatory alopecia types remain unclear. This case report evaluated the effectiveness of MN in the treatment of alopecia in dogs with endocrine disorders. The study included one dog each with hypothyroidism (Case-1), hyperadrenocorticism (Case-2), and alopecia X (Case-3). Despite controlled hormone levels, dogs of Cases 1 and 2 showed no hair regrowth. Microneedling was performed on alopecic lesions, and hair regrowth was monitored at 1, 3, 6, and 12 months. In Case-1, almost complete hair regrowth was observed at 12 months. In Case-2, most lesions exhibited regrowth at 6 months, with no hair loss up to 12 months. Case-3 exhibited 51–75% regrowth at 12 months. These findings suggest that MN may serve as an adjunct treatment for dogs with controlled hormone levels but persistent alopecia.

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#### INTRODUCTION

Non-inflammatory alopecia resulting from aberrant hair follicle cycling in dogs is commonly associated with endocrine diseases such as hyperadrenocorticism, hypothyroidism, sex hormone imbalance, and alopecia X or Black Skin Disease (Welle, 2023). Although the clinical manifestations of endocrine diseases can be managed by restoring hormone balance, dermatological responses are often slower, and hair loss may persist even after other systemic symptoms disappear with treatment (Bugbee *et al.*, 2023).

Microneedling (MN) is a relatively new minimally invasive treatment that uses a device called a dermaroller to create microwounds in the skin, stimulating wound healing and promoting hair regrowth (Zhou et al., 2023). In humans, MN is effective in treating various types of alopecia, including androgenic alopecia, alopecia areata, and female pattern baldness (Zhou et al., 2023). Similarly, microneedles have shown promising therapeutic effects in dogs with alopecia X (Stoll et al., 2015). Moreover, a recent study reported that needle length is a critical factor in hair follicle regeneration, with longer needles potentially being more effective in promoting hair regrowth (Kang et al., 2024). However, data on the

efficacy of MN for other types of non-inflammatory alopecia in dogs remain limited. It was hypothesized that microneedling would be effective for hair growth in non-inflammatory alopecia of endocrine origin. Therefore, the present study was planned to investigate the effects of MN using a dermaroller with a long needle length (2.5mm) to promote hair regrowth in dogs with non-inflammatory alopecia, including cases associated with hormonal disorders.

#### MATERIALS AND METHODS

Ethical approval: All procedures in this study were approved by the Institutional Animal Care and Use Committee of Jeonbuk National University (approval number: NON2024-167-001). Owners were fully informed of the study procedures, and written consent was obtained from each owner before enrollment of the dog for this study.

**History and clinical signs:** This study included three dogs, the first with hypothyroidism, the second with hyperadrenocorticism, and the third with suspected alopecia X. Case-1 involved a 10-year-old castrated male Pomeranian diagnosed with hypothyroidism based on low total T4 levels  $(0.971 \mu g/dL)$ ; reference range  $1-4\mu g/dL$ ,

IDEXX Laboratories, Westbrook, ME, USA) and free T4 levels (<0.30ng/dL; reference range 0.6–3.7ng/dL, IDEXX Laboratories), along with clinical signs of decreased vigor. First onset of alopecia was seen when the dog was 9 years, 8 months old. The dog had been treated with levothyroxine (0.02mg/kg/day) for four months, and total T4 levels were controlled (3.5 $\mu$ g/dL; therapeutic range, 2.1–5.4 $\mu$ g/dL, IDEXX Laboratories). While the clinical signs of decreased vigor resolved, alopecia on the trunk, flanks, and caudal thighs persisted.

Case-2 involved a 10-vear-old castrated male Pomeranian diagnosed with hyperadrenocorticism based on an adrenocorticotropic hormone (ACTH) stimulation test (post-ACTH cortisol: 23.69µg/dL; reference range 6-18µg/dL, IDEXX Laboratories) and bilateral adrenal gland enlargement was observed on ultrasonography, with each gland measuring 0.6cm, as shown in Fig 1A (left gland) and in Fig. 1B (right gland). First onset of alopecia in this dog was seen at 6-years of age. The dog exhibited polyuria, polydipsia, and polyphagia. It had been treated with trilostane (2.2mg/kg/day) for four years, and post-ACTH cortisol levels (3.95µg/dL; therapeutic range, 1.5– 5.4µg/dL, IDEXX Laboratories) remained controlled. While polyuria, polydipsia, and were polyphagia resolved, hair regrowth was not observed over four years. The dog was also administrated with melatonin (6mg/day) for 1 month, but no hair regrowth was observed. Total T4 levels was also within reference range (1.1µg/dL; reference range 1-4µg/dL, IDEXX Laboratories).

Case-3 involved a 2-year-old castrated male Pomeranian that first developed alopecia at one year of age. Total T4 levels  $(1.2\mu g/dL)$ ; reference range  $1-4\mu g/dL$ ) were within the reference range, and no clinical signs of endocrine disease, such as polyuria or polydipsia, were observed. Based on the age of onset and the exclusion of other endocrine disorders, the dog was suspected to have alopecia X.

**Microneedling application:** All dogs were bathed with 4% chlorhexidine shampoo two days before the MN procedure. The MN procedure was performed during the recovery phase of general anesthesia using isoflurane for some other problem. The affected area was disinfected with 2% chlorhexidine and treated with 2.5% lidocaine and 2.5% prilocaine cream (0.1g/cm², EMLA, Mitsubishi

Tanabe). A punch biopsy was performed in the affected area in Case-2 and Case-3, as the owner of Case-1 did not agree to perform skin biopsy of his dog. Microneedling procedure was conducted in all three dogs. Accordingly, a dermaroller with 2.5-mm needles (MTS, ZGTS) was applied to the dorsum, flank, and posterior thigh in horizontal, vertical, and diagonal directions for 10 min each. After the procedure, the treated area was disinfected again with 2% chlorhexidine, and cefovecin (8mg/kg) was administered subcutaneously. The levothyroxine (0.02mg/kg/day) in Case 1 and the trilostane (2.2mg/kg/day) in Case-2 were administered for 12 months after MN procedure.

# Evaluation of the effectiveness of the MN procedure: Subjective hair regrowth was evaluated in three dogs at 1, 3, 6, and 12 months after MN procedure, as previously reported (Kang *et al.*, 2024). Accordingly, the same veterinarian grossly evaluated hair regrowth during 12 months after MN. A five-point scale (0-4) was used, as described below: 0: no improvement; 1: 1–25% improvement; 2: 26–50% improvement; 3: 51–75% improvement; and 4: 76–100% improvement. Hair regrowth rates were assessed for the entire body and

separately for different body sites, including the back, flank, and thigh. Adverse reactions, if any, were recorded throughout the 12-month follow-up period.

#### **RESULTS**

Histopathological findings of alopecia lesions in Case-2 and Case-3 showed hair follicles in the telogen phase. Hair regrowth after MN procedure was evaluated in three dogs for 12 months. After 1 month of MN procedure, all three dogs showed 1-25% hair regrowth. However, Case-1 showed an increase in hair regrowth (51-75%) after 6 months and achieved nearly complete regrowth at 12 months (Fig. 2A). In Case-2, 76–100% hair regrowth was observed after 6 months, and the dog maintained the regrown hair without recurrence of hair loss until 12 months post-procedure. Case-3 exhibited less than 50% regrowth at 3 and 6 months, with 51–75% regrowth was observed at 12 months (Fig. 2A). Additionally, improvements in hair coat quality and overall hair regrowth were noted in all three dogs.





Fig. 1: Ultrasonography of adrenal glands in Case-2, showing bilateral enlargements of adrenal glands: A); left adrenal gland (arrows), measuring 0.6cm, B); right adrenal gland (arrows), measuring 0.6cm.

Hair regrowth was assessed at different anatomical sites, including the dorsal trunk, flank, and thigh. One month after MN procedure, hair regrowth was mainly observed on the dorsal trunk, suggesting that hair regrowth occurred most rapidly in this region of Case-2 (Fig. 2C), Case-3 (Fig. 2D). In Case-1, besides the dorsal trunk, some hair growth was also seen in the flank and thigh regions (Fig. 2B). In Case-1 (Fig. 2B) and Case-2 (Fig. 2C), 51-75% hair regrowth was observed on the dorsal trunk at 3 months, with some growth (25-50%) in the flank and thigh regions in all three dogs at this stage (Fig. 2B-D). About 51-75% hair regrowth was observed in all three regions in Case-1 (Fig. 2B) and Case-2 (Fig. 2C) at 6 months. At 12 months, 76-100% hair regrowth was observed in all three regions of Case-1 (Fig. 2B) and Case -2 (Fig. 2C). However, in Case-3, 51-75% hair growth was seen in dorsal trunk and 26-50% in flank and thigh regions 12 months after MN procedure (Fig. 2D).

The clinical manifestations in Cases 1–3 are shown in Fig. 3. Nearly complete hair regrowth was observed in Case-1 (Fig. 3A and B) and Case-2 (Fig. 3C and D) at 12 months, whereas Case-3 exhibited <75% regrowth (Fig. 3E and F). Adverse effects were evaluated in all three dogs at 12 months post-procedure. In Case-2, MN induced intermittent generalized scaling and malodor for 6 months. In Cases-1 and 3, generalized erythema was observed following MN procedure but resolved within 1 week. Skin malodor persisted for 1 month, and generalized scaling was observed for approximately 1–3 months. No systemic signs, such as anorexia or lethargy, were observed in any of the dogs.

#### DISCUSSION

In this case report, microneedling (MN) procedure induced hair regrowth in three dogs with noninflammatory alopecia. In particular, Case-1 and Case-2 diagnosed were with endocrine disorders hypothyroidism and hyperadrenocorticism, respectively. Although hormone levels were within the therapeutic range at the time of MN application and other clinical signs had been resolved as a result of hormonal treatment, hair regrowth was not observed. Following MN treatment. both Case-1 and Case-2 exhibited hair regrowth within 6-12 months, despite unchanged levothyroxine and trilostane doses. These findings suggest that MN procedure may be a potential treatment for alopecia in dogs with controlled hormone levels but persistent hair loss.

Hypothyroidism, hyperadrenocorticism, and alopecia X are forms of non-inflammatory alopecia characterized by aberrant hair follicle cycling (Müntener *et al.*, 2013). Hair in alopecic areas remain in the telogen phase, indicating that hair loss is caused by impaired telogen-to-anagen transition (Choi, 2020). Previous studies have shown that microneedle therapy promotes hair regeneration by activating the Wnt/β-catenin pathway, a key regulator of telogen-to-anagen transition (Fertig *et al.*, 2018; Pei *et al.*, 2022; Zhou *et al.*, 2023). The Wnt/β-catenin pathway facilitates this transition by promoting the migration and proliferation of outer root sheath cells, hair matrix cells, and dermal papilla cells (Choi, 2020).

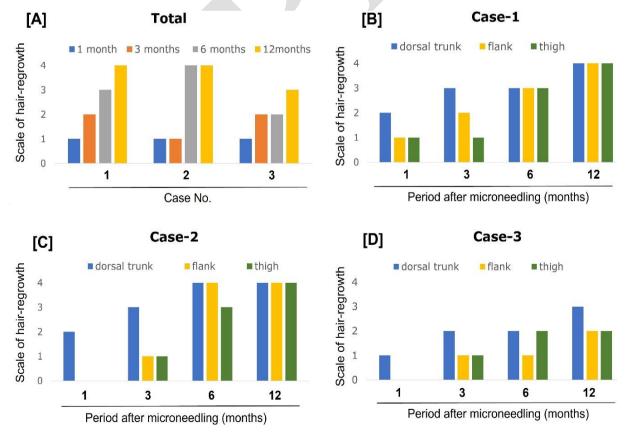


Fig. 2: Hair regrowth rate in Cases I–3: A). Total hair growth on dorsal trunk, flank and thigh area in each case; B). Hair growth at three areas at different times after MN procedure in Case-1; C). Hair growth at three areas at different times after MN procedure in Case-2; D). Hair growth at three areas at different times after MN procedure in Case-3. Scale of hair-regrowth: 0: no improvement; 1: I–25% improvement; 2: 26–50% improvement; 3: 51–75% improvement; and 4: 76–100% improvement.



**Fig. 3:** Clinical manifestations in Cases I–3. A). Before MN procedure, alopecia was observed on the dorsal trunk, flank, and thigh in Case-I. B). At I2 months after MN procedure, almost complete hair regrowth was observed in Case-I. C). In Case-2, alopecia was present on the neck, dorsal trunk, flank, limbs, and thigh. D). At I2 months after MN procedure, hair regrowth was observed in most alopecic areas in Case-2. E). Case-3 also showed alopecia on the dorsal trunk, flank, and thigh. F). At I2 months after MN procedure, hair regrowth was primarily observed on the dorsal trunk, with partial regrowth on the flank and thigh in Case-3.

Additionally, during the wound-healing process, MN procedure stimulates the production of growth factors, including platelet-derived growth factor, epidermal growth factor, and vascular endothelial growth factor, thereby increasing blood supply to hair follicles (Pei *et al.*, 2022). These mechanisms suggest that in the three dogs included in this study, MN procedure may have reactivated the arrested hair cycle, inducing hair follicles to enter the anagen phase.

In most cases, the rate of hair regrowth on the dorsal trunk was higher than that on the flank and thigh regions. This finding suggests that, despite the procedure being performed by the same practitioner with consistent pressure, the flank and thigh regions, which have greater curvature than the dorsal trunk, may not have received the same level of pressure. Further research is needed to assess the relationship between hair regrowth rate and pressure distribution across different body sites.

In humans, microneedles are available in solid, coated, dissolvable, and hydrogel-forming forms (Fertig *et al.*, 2018; Zhou *et al.*, 2023). MN in combination with topical drugs application enhances the transdermal absorption of therapeutic agents, leading to greater hair regrowth than MN procedure alone (Pei *et al.*, 2022). Previous studies in humans have demonstrated that MN procedure combined with hair growth-promoting agents, such as minoxidil, platelet-rich plasma, and topical corticosteroids, further enhances hair regrowth (Pei *et al.*, 2022). In veterinary medicine, a previous study reported that MN combined with platelet-rich plasma improved hair regrowth in dogs with post-clipping alopecia (Diamond *et al.*, 2020). Future studies exploring various microneedle formulations in combination with topical

agents could offer a novel treatment approach for dogs with non-inflammatory alopecia.

This study had a few limitations. hypothyroidism, hyperadrenocorticism, and alopecia X were each evaluated in only one dog. Future clinical trials with larger sample size are warranted. Second, both dogs with hormonal disorders were Pomeranians, making it impossible to completely rule out concurrent alopecia X. However, alopecia in these two dogs first appeared in middle to old age, and clinical signs of alopecia were accompanied by other hormonal-imbalance related symptoms. such as decreased vigor polyuria/polydipsia, suggesting a strong association between alopecia and endocrinopathy. Third, because follow-up was conducted for only 12 months after MN procedure in this study, hair loss recurrence could not be assessed. Future studies with longer follow-up periods are needed to evaluate long-term treatment success.

Conclusions: In conclusion, MN procedure induced significant hair regrowth in dogs with non-inflammatory alopecia, including those with hormonal disorders. Hence, MN could be considered as an additional treatment option in clinical settings when alopecia does not improve despite ongoing endocrine disease management.

**Author's contribution:** YY, WCK, and SO contributed to the management of the cases. YY, JYK, JP, and JSY contributed to the data collections. YY, YJ, JP, and JSY contributed to the writing of the manuscript. All authors have read and approved the final version of the manuscript.

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