# Enhanced Effect of Platelet-Rich Plasma Containing a New Carrier on Hair Growth

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BACKGROUND Treatments for alopecia are in high demand, but not all are safe and reliable. Dalteparin and protamine microparticles (D/P MPs) can effectively carry growth factors (GFs) in platelet-rich plasma (PRP).

OBJECTIVE To identify the effects of PRP-containing D/P MPs (PRP&D/P MPs) on hair growth.

METHODS & MATERIALS Participants were 26 volunteers with thin hair who received five local treatments of 3 mL of PRP&D/P MPs (13 participants) or PRP and saline (control, 13 participants) at 2- to 3-week intervals and were evaluated for 12 weeks. Injected areas comprised frontal or parietal sites with lanugo-like hair. Experimental and control areas were photographed. Consenting participants underwent biopsies for histologic examination.

RESULTS D/P MPs bind to various GFs contained in PRP. Significant differences were seen in hair cross-section but not in hair numbers in PRP and PRP&D/P MP injections. The addition of D/P MPs to PRP resulted in significant stimulation in hair cross-section. Microscopic findings showed thickened epithelium, proliferation of collagen fibers and fibroblasts, and increased vessels around follicles.

CONCLUSION PRP&D/P MPs and PRP facilitated hair growth but D/P MPs provided additional hair growth.

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Platelet-rich plasma (PRP) contains a high concentration of thrombocytes. PRP carries various growth factors (GFs) that stimulate cell proliferation and differentiation. PRP is known to carry more than 20 GFs, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF), almost all of which are known to be heparin-binding. Recent work has demonstrated the role of PRP in bone grafts, teeth osteosynthesis, and wound healing.[1–5] In 2006, Uebel and colleagues reported a new application of PRP for male pattern baldness surgery.[6] Implanting follicular units with PRP raised the hair yield rate, probably because of the partial effects of GFs in PRP. The action of GFs on the germinative hair cycle has been studied in embryologic and adult phases,[7–9] but no clinical trial or experimental protocol has previously verified the efficacy of those factors in the growth and density of hair after subcutaneous injection into a thinning area.

We have previously reported dalteparin and protamine microparticles (D/P MPs) as a carrier for controlled release of GFs such as FGF-2.[10,11] The material consists of a low-molecular-weight heparin (dalteparin) with protamine, resulting in water-insoluble microparticles approximately 0.5 to 3 μm in diameter. FGF-2-containing D/P MPs show a substantial ability to induce vascularization and

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fibrous tissue formations.[10,11] PRP contained higher amounts of various GFs to stimulate proliferation of human microvascular endothelial cells and dermal fibroblast cells, and immobilized GFs on the D/P MPs appeared to be bioactive (unpublished data). Furthermore, D/P MPs were able to protect activity of GFs in PRP from inactivation by incubation at 37°C for longer than 7 days (unpublished data). These results demonstrated that GFs in PRP are bound to and stabilized on D/P MPs and that GFs incorporated onto D/P MPs are gradually diffused and released upon biodegradation of D/P MPs in vivo. The present study demonstrated that PRP carries high amounts of platelets that carry various GFs and that a majority of those GFs can be immobilized on D/P MPs. A clinical trial using PRP-containing D/P MPs (PRP&D/P MPs) was developed to investigate whether administration of autologous PRP&D/P MPs could be used as a new treatment for alopecia.

## Materials and Methods

### *Preparation of D/P MPs*

The production of D/P MPs has been described previously.[10] Briefly, 0.3 mL of protamine solution (Mochida Pharmaceutical Co., Tokyo, Japan) was added dropwise to 0.7 mL of dalteparin solution (Kissei Pharmaceutical Co., Tokyo, Japan) with hard vortexing for approximately 2 minutes. To maximize the production of MPs, protamine and dalteparin were mixed in a ratio at 3:7 (vol:vol). The solution of D/P MPs (1 mL) was then washed twice with phosphate-buffered saline by centrifugation to remove unreacted components, and the volume was filled to 1 mL with phosphate-buffered saline. Approximately 6 mg of the dry D/P MPs was obtained from 1 mL of the D/P MP solution.

### *Preparations of PRP and PRP&D/P MPs*

PRP was prepared as previously described.[10,11] Briefly, 15 mL of blood from each volunteer was drawn into a tube containing 1.5 mL of 2% sodium citrate (Nipro Pharma, Osaka, Japan). The tubes were centrifuged for 15 minutes at 1,700 revolutions per minute (rpm) (Table-Top Refrigerated Centrifuge 2800, Roter: RS-240, Kubota Corp., Tokyo, Japan), resulting in three basic layers: an erythrocyte layer at the bottom of the tube, a PRP layer in the middle, and a platelet-poor plasma (PPP) layer at the top of the tube. The upper 1 cm of the red blood cell layer (PRP layer) was collected and centrifuged for 5 minutes at 3,000 rpm to concentrate the platelets. The PRP was frozen at −80°C until usage. The 2 mL of thawed PRP obtained was mixed with 1 mL of D/P MPs to prepare PRP&D/P MPs. Finally, the PRP&D/P MPs used in this clinical study consisted of frozen and thawed 67% PRP and 2 mg/mL D/P MPs. In this procedure, 4 mL of PRP and 16 mL of PPP were obtained from 40 mL of blood. The platelets in PRP and whole blood were microscopically counted. Platelet concentration in PRP ($88.2 \pm 21.7 \times 10^4$/1 μL, $n = 15$ persons) was significantly higher than that in whole blood ($14.4 \pm 3.8 \times 10^4$/1 μL, $n = 15$ persons).

### *Enzyme-Linked Immunosorbent Assay for Evaluating the Binding of GFs in PRP to D/P MPs*

Enzyme-linked immunosorbent assays (ELISAs) for GFs in PRP or PPP were performed to evaluate the adsorption of those GFs to the D/P MPs. One milliliter of D/P MPs (6 mg/mL) in saline was added to 2 mL of prepared PRP and incubated for 2 hours at room temperature on a rotary shaker. The mixtures were then centrifuged to remove the GF-containing D/P MPs (GF&D/P MPs). The amount of each GF in the diluted supernatants was measured using an ELISA kit (R&D Systems, Inc., Minneapolis, MN) for FGF-2, HGF, epidermal growth factor (EGF), keratinocyte growth factor, VEGF, insulin-like growth factor-1 (IGF1), PDGF-AB, PDGF-BB, TGFβ1, and TGFβ2 according to the manufacturer's instructions.

### *Participants*

Participants were 26 individuals (16 men, 10 women) aged 28 to 59 with thin hair in the frontal or parietal areas. Injection sites were the desired sides, and control sites were the opposing side or a part showing grossly equal density of hair. The distances from nasal

tip and upper part of the auricular base to the injected sites were measured, and the same locations were thus able to be selected accurately whenever needed (Figure 1). Participants were photographed using a digital camera (Nikon, Tokyo, Japan) and a dermoscopic digital camera (Derma Medical, Kanagawa, Japan), and the PRP or PRP&D/P MPs were administered (Figure 1). All research protocols were submitted and approved by the Ethics Committee of the National Defense Medical College, and each participant was informed and signed a written consent form before participating in this study.

### *Administration of PRP&D/P MPs and Evaluation*

Participants received a single subcutaneous injection of 3 mL of PRP&D/P MPs (67% PRP with 2 mg/mL of D/P MPs in saline) or PRP (67% with saline) into the skin of the scalp using a 25-G needle. As a control, 3 mL of saline was injected into the opposing side to the experimental side. Participants received five injections, at weeks 0, 2, 4, 6, and 9, and were observed for 12 weeks. The areas administered PRP&D/P MPs or PRP and the control areas were photographed using a digital camera and a dermoscopic digital camera. The number of all hairs in a 1.0- × 1.0-cm area was counted in dermoscopic images, and diameters of all hair shafts were measured. Mean cross-sections of all hairs in the 1-cm$^2$ area were calculated using the measured data.

### *Histologic Examination*

Biopsies were performed from consenting participants before first injection and after the fifth injection of PRP&D/P MPs or PRP (after 12 weeks) using a 4-mm disposable biopsy punch (Kai Industries, Gifu, Japan) under anesthesia with 1% lidocaine hydrochloride with epinephrine (Astra Zeneca, Osaka, Japan). The biopsy specimens were fixed in 10% formalin solution (Wako Pure Chemical Industries, Osaka, Japan) and embedded in paraffin. Paraffin blocks were sectioned in 4-μm increments and stained with hematoxylin and eosin (H&E) at the Technical Service Center at SRL (Tokyo, Japan).

### *Statistical Analyses*

Data are provided as means ± standard errors of the mean (SEM). One-way repeated-measures analysis of variance was used to compare PRP&D/P MPs-treated, PRP-treated, and control areas. Statistical

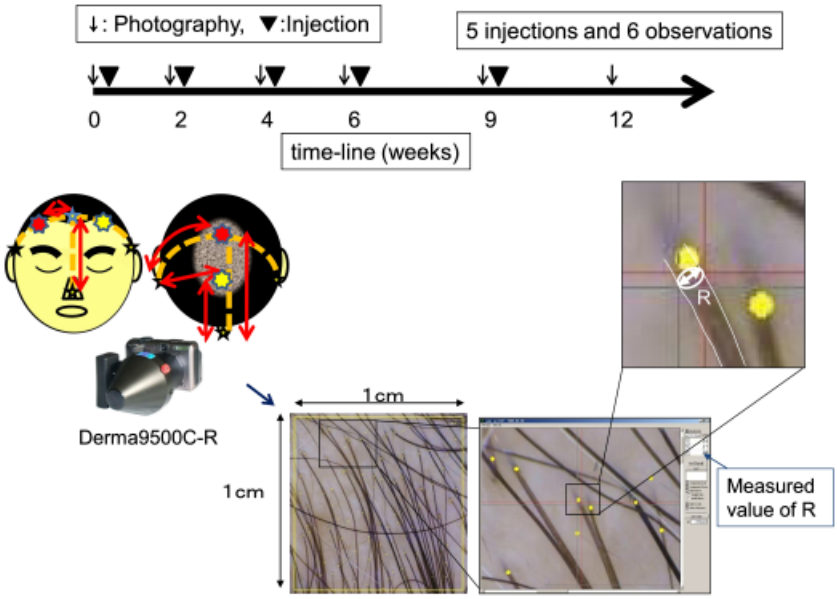


**Figure 1.** Illustration of experimental procedure. Participants received five injections and were observed for 12 weeks. R, diameter of hair shaft.

analyses were performed using StatMate III for Windows (ATMS, Tokyo, Japan). Probability values of less than 0.05 were accepted as significant.

## Results

### GFs in PRP and Adsorption of GFs to D/P MPs

All tested GFs except EGF and IGF in PRP are known to bind specifically to heparin. Those GFs in PRP and PPP were measured using ELISA methods as described above. The amounts of GFs in frozen and thawed PRP and PPP were summarized in Table 1. These results indicated that roughly 31% to 100% of each GF in whole blood plasma (PRP + PPP) were concentrated in PRP and that concentrations of various GFs in PRP were higher than in whole plasma and PPP.

When 0.33 mL of D/P MPs in saline (6 mg/mL) was added to 0.67 mL of PRP, incubated at room temperature for 2 hours, and centrifuged to separate the GF&D/P MPs, there were significantly fewer GFs, except EGF and IGF, in the supernatants, indicating that significant parts of those GFs were adsorbed onto D/P MPs (Table 2).

**TABLE 2. Growth Factors in 1 mL of 67% Platelet-Rich Plasma Absorbed by 2 mg of Dalteparin and Protamine Microparticles**

| *Growth Factor* | *Amount Adsorbed* | *%* |
|---|---|---|
| Fibroblast growth factor-2, pg | 12.2 | 86 |
| Hepatocyte growth factor, pg | 244 | 83 |
| Epidermal growth factor, pg | 6.2 | 19 |
| Keratinocyte growth factor, pg | 148 | 84 |
| Vascular endothelial growth factor, pg | 282 | 90 |
| Insulin-like growth factor-1, ng | 222 | 19 |
| Platelet-derived growth factor, ng | | |
| AB | 285 | 86 |
| BB | 1.7 | 94 |
| Transforming growth factor, ng | | |
| β1 | 1.39 | 98 |
| β2 | 1.08 | 94 |

### Administration of PRP&D/P MPs and Evaluation

Mean numbers of hairs were 104 ± 6, 112 ± 6, and 114 ± 6/cm$^2$ at the experimental site (1 cm$^2$) before administration of control, PRP, and PRP&D/P MPs, respectively. After 12 weeks, when participants had received all five treatments, mean numbers of hairs were 106 ± 5, 127 ± 6, and 132 ± 7/cm$^2$, respectively (Table 3, Figures 2–4). Rates of increase were 1.9%, 13.4%, and 15.8%, respectively. Although there were no significant differences between the PRP&D/P MP- and PRP-injected groups, both exhibited a greater mean number of hairs than the control group.

**TABLE 1. Growth Factors (GFs) in 67% Platelet-Rich Plasma (PRP) and Platelet-Poor Plasma (PPP)**

| *GF* | *PRP, mean ± SEM* | *GFs in PRP, %* | *PPP, mean ± SEM* |
|---|---|---|---|
| Fibroblast growth factor-2, pg/mL | 14.4 ± 2.9 | 78 | 1.0 ± 0.5 |
| Hepatocyte growth factor, pg/mL | 294.8 ± 12.2 | 31 | 163.6 ± 11.7 |
| Epidermal growth factor, pg/mL | 32.2 ± 3.6 | 66 | 4.2 ± 1.0 |
| Keratinocyte growth factor, pg/mL | 177.4 ± 40.1 | 77 | 13.1 ± 2.4 |
| Vascular endothelial growth factor, pg/mL | 312.2 ± 43.9 | 33 | 162.8 ± 5.0 |
| Insulin-like growth factor-1, ng/mL | 1.2 ± 0.1 | 71 | 0.12 ± 0.02 |
| Platelet-derived growth factor, ng/mL | | | |
| AB | 331.8 ± 92.5 | 100 | N/D |
| BB | 1.8 ± 0.3 | 72 | 0.17 ± 0.08 |
| Transforming growth factor, ng/mL | | | |
| β1 | 1.4 ± 0.1 | 100 | N/D |
| β2 | 1.1 ± 0.4 | 100 | N/D |

SEM, standard error of the mean; N/D, not determined.

**TABLE 3. Hair Counts and Hair Cross-Sections Before and After Administration of Platelet-Rich Plasma (PRP) and PRP-Containing Dalteparin and Protamine Microparticles (PRP&D/P MPs) ($n = 13$)**

| *Control* | | | | *PRP Only* | | | | *PRP&D/P MPs* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Hairs per 1.0 cm²* | | *Hair Cross-section ($\times 10^{-4}$ mm²)* | | *Hairs per 1.0 cm²* | | *Hair Cross-section ($\times 10^{-4}$ mm²)* | | *Hairs per 1.0 cm²* | | *Hair Cross-section ($\times 10^{-4}$ mm²)* | |
| *Before* | *After* | *Before* | *After* | *Before* | *After* | *Before* | *After* | *Before* | *After* | *Before* | *After* |
| 76 | 79 | 7.3 | 8.4 | 71 | 92 | 8.4 | 11.5 | 78 | 95 | 5.5 | 14.0 |
| 123 | 129 | 12.0 | 12.0 | 126 | 138 | 11.5 | 15.9 | 141 | 173 | 11.5 | 18.5 |
| 118 | 122 | 6.6 | 6.0 | 131 | 147 | 7.5 | 11.5 | 124 | 146 | 6.5 | 12.7 |
| 139 | 135 | 6.8 | 7.4 | 155 | 160 | 6.5 | 13.5 | 154 | 175 | 5.9 | 11.5 |
| 72 | 81 | 2.9 | 4.4 | 101 | 105 | 4.4 | 7.5 | 85 | 98 | 5.0 | 9.8 |
| 94 | 91 | 3.9 | 3.0 | 97 | 102 | 4.4 | 6.5 | 110 | 119 | 6.0 | 11.5 |
| 89 | 92 | 3.8 | 3.4 | 93 | 109 | 4.5 | 5.8 | 45 | 55 | 3.0 | 9.8 |
| 111 | 115 | 7.0 | 6.0 | 121 | 138 | 3.4 | 4.9 | 144 | 155 | 3.8 | 10.0 |
| 141 | 144 | 6.5 | 6.4 | 102 | 141 | 6.4 | 8.1 | 101 | 128 | 3.7 | 10.5 |
| 102 | 102 | 4.5 | 3.0 | 104 | 109 | 7.5 | 9.5 | 124 | 143 | 3.9 | 10.0 |
| 89 | 92 | 5.5 | 6.0 | 106 | 132 | 4.5 | 10.5 | 128 | 142 | 3.9 | 9.5 |
| 85 | 84 | 3.5 | 4.4 | 128 | 140 | 4.3 | 5.5 | 129 | 148 | 3.8 | 9.2 |
| 114 | 110 | 3.1 | 3.4 | 116 | 136 | 4.4 | 5.5 | 114 | 137 | 3.7 | 8.4 |
| Mean ± SEM | | | | | | | | | | | |
| 104 ± 6 | 106 ± 6 | 5.6 ± 0.7 | 5.7 ± 0.7 | 112 ± 6 | 127 ± 6 | 6.0 ± 0.7 | 8.9 ± 1.0 | 114 ± 7 | 132 ± 7 | 5.1 ± 0.6 | 11.2 ± 0.8 |

SEM, standard error of the mean.

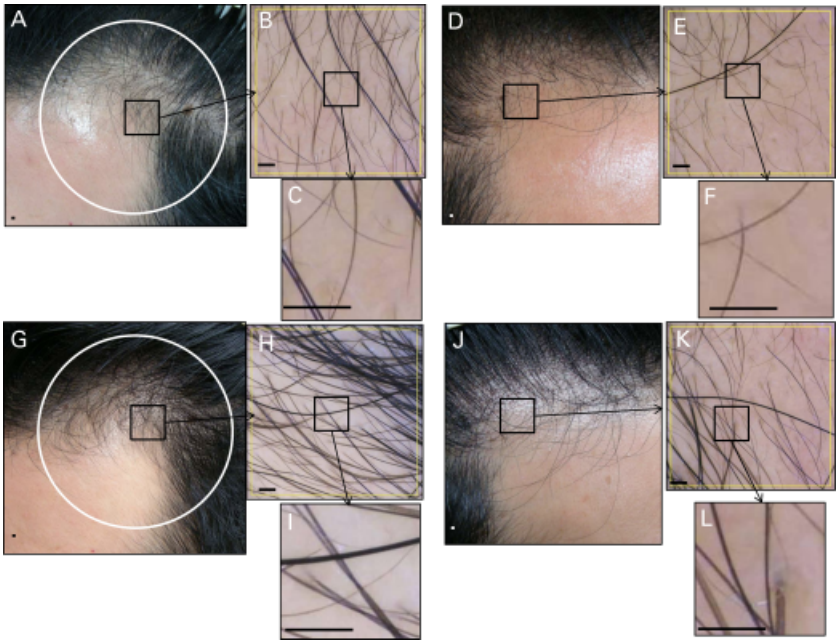


**Figure 2.** A 38-year-old man with regions of frontal thin hair. (A–F) Before administration of platelet-rich plasma–containing dalteparin and protamine microparticles (PRP&D/P MPs) (A–C) and saline (D–F). (G–L) After 12 weeks of administrations of PRP&D/P MPs (G–I) and saline (J–L). White circles show the experimental areas. Scale bar = 1 mm.

Mean cross-sections of hairs were $5.6 \pm 0.7$, $6.0 \pm 0.7$, and $5.1 \pm 0.6$ ($\times 10^{-4}$ mm$^2$) in the experimental site (1 cm$^2$) before examination of control, PRP, and PRP&D/P MPs, respectively, in both areas. At the endpoint (12 weeks after first injection) of evaluation at the fifth injection, they were $5.7 \pm 0.7$, $8.9 \pm 1.0$, and $11.2 \pm 0.8$ ($\times 10^{-4}$ mm$^2$), respectively (Table 3, Figures 2–4). Rates of increase after the fifth injection (at 12 weeks) were 1.8%, 48.3%, and 119.6%, respectively. Significant differences were seen after the fifth injection (at 12 weeks) between the control group and the PRP and PRP&D/P MP groups ($p < .01$, Table 3, Figure 4).

Subjective effects of PRP&D/P MPs and PRP according to participants were less depilation when shampooing, greater bounce and resilience of hair, and maintenance of healthy hairs. Eight cases in the PRP&D/P MP group were more than 100% greater in mean cross-sections, and the others (5 cases) were 50% to 100% greater. In contrast, only two cases in the PRP group were more than 100% increased, two cases were 50% to 100% increased, and nine cases were 10% to 50% increased; no participants showed deterioration. All participants experienced temporary pain at the injection site but no side effects with either injection, such as hematoma or infection.

Consenting participants were histologically examined before the first injection and after the fifth injection (12 weeks). Head skin was biopsied and stained with H&E reagent (Figure 5). After administration of PRP&D/P MPs and PRP, microscopic findings showed thickened epithelium, proliferation of collagen fibers and fibroblasts, and greater numbers of blood vessels around hair follicles.

## Discussion

We have previously reported that PRP contains higher concentrations of various GFs than PPP and that D/P MPs effectively adsorb those GFs. Microvascular endothelial cells and dermal fibroblast cells were optimally grown with medium containing less than 2% PRP, and addition of D/P MPs significantly enhanced and protected the proliferative activity of

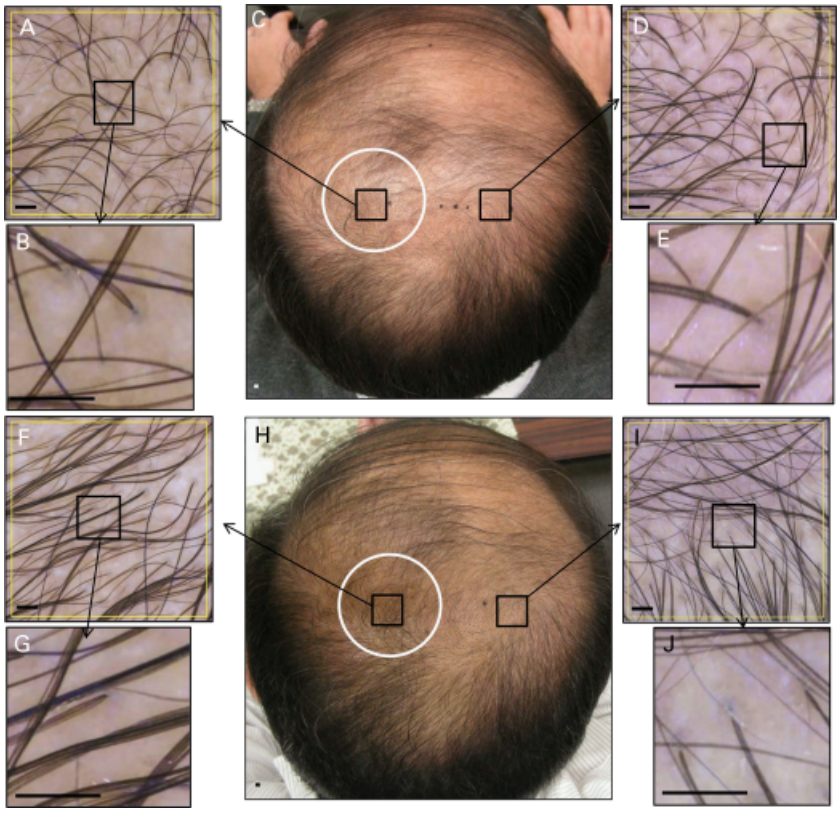


**Figure 3.** A 54-year-old man with regions of parietal thin hair. (A–E) Before administration of platelet-rich plasma–containing dalteparin and protamine microparticles (PRP&D/P MPs) (A–C) and saline (C–E). (F–J) After 12 weeks of administration of PRP&D/P MPs (F–H) and saline (H–J). White circles show the experimental areas. Scale bar = 1 mm.

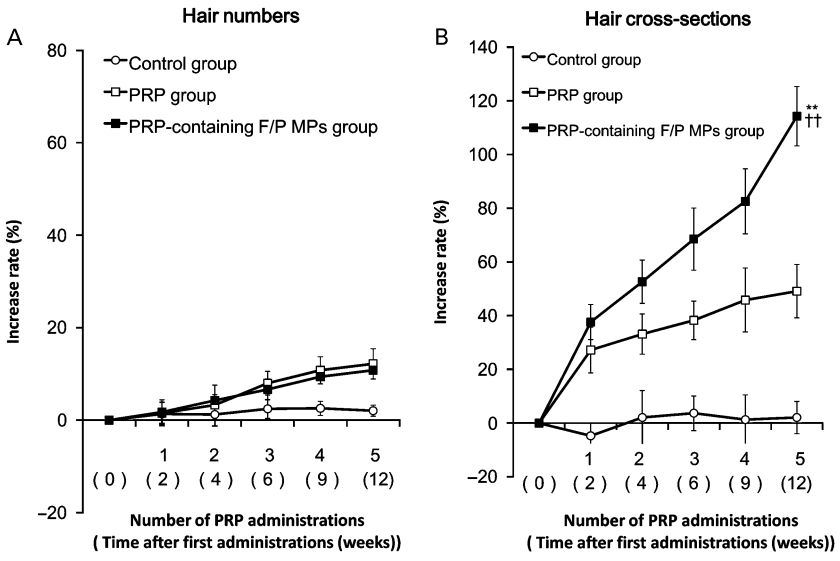


**Figure 4.** Hair numbers and cross-sections before and after administration of platelet-rich plasma (PRP)-containing dalteparin and protamine microparticles (PRP&D/P MPs), PRP alone, and saline alone (control). $^{*}p<.05$, $^{**}p<.01$ compared with control and $^{\dagger\dagger}p<.01$ compared with PRP alone.

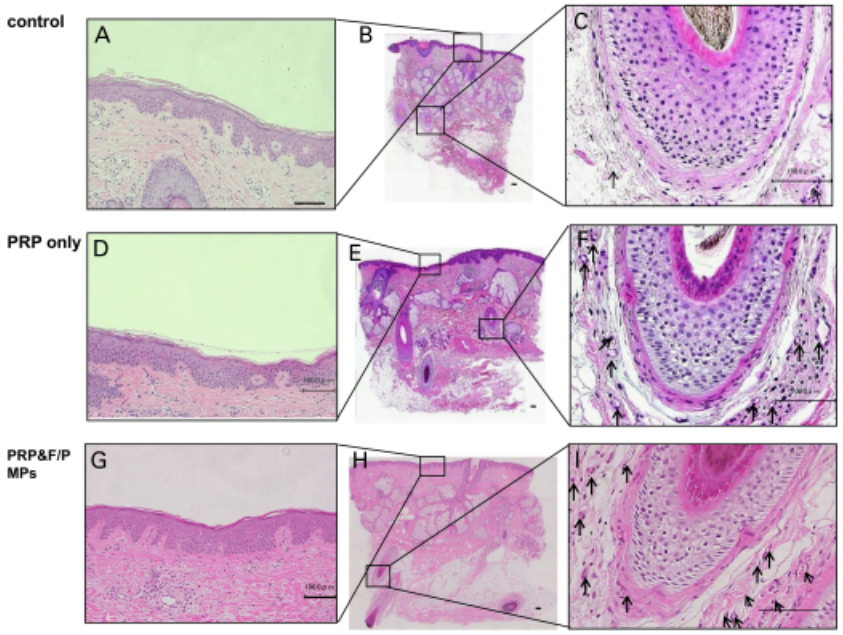


**Figure 5.** Microphotographs of a 30-year-old man with a region of frontal thin hair with hematoxylin and eosin staining. Original magnification: × 100 (A, D, G), × 15 (B, E, H), × 200 (C, F, I). (A–C) A region of frontal baldness treated with saline (control) five times after 12 weeks. (D–F) A region of frontal baldness administrated with platelet-rich plasma (PRP) alone. (G–I) A region of frontal baldness administrated with PRP-containing dalteparin and protamine microparticles (PRP&D/P MPs). (C, F, I) An arrow shows vessels around fair follicle. Many vessels were seen in PRP- and PRP&D/P MPs-injected regions. Scale bar = 100 μm.

PRP in vitro in lower concentrations (<1%) for more than 10 days (unpublished data). In the present study, platelets in plasma were concentrated in PRP. More than 80% of those GFs in PRP except EGF and IGF-1 (which are known to be non-heparin-binding GFs) were immobilized onto the D/P MPs. Because only concentrations of GFs in PRP were determined using indirect ELISA used in this study, the concentrations determined may not correlate directly with the activities of GFs, although the results together with previous (above) results suggest that GFs in PRP with platelets are bound to and stabilized on D/P MPs and that GFs, just like FGF-2 incorporated in D/P MPs, are gradually diffused and released upon biodegradation of D/P MPs in vivo.[10,11] We previously reported that FGF-2 immobilized onto D/P MPs was released with a half-releasing time of approximately 7 days in vitro.[10,11]

GFs contained in PRP are released from α-granules of thrombocytes. The α-granules of platelets include various GFs and other bioactive proteins, including VEGF, IGF-1, HGF, PDGF, TGF, EGF, platelet factor-4, interleukin-1, platelet-derived angiogenesis factor, platelet-derived endothelial growth factor, and epithelial cell growth factor.[1–5] In recent research, various GFs have been found to act on hair follicles as regulators of hair growth and cycle.[12–16] In addition, D/P MPs may be able to adsorb various heparin-binding substances involved in cell proliferation, migration, and angiogenesis, such as GFs and cytokines in PRP.[11,12] This study showed that those various GFs were concentrated in PRP (Table 1), and direct local administration of those GFs in PRP may act on hair follicles and indirectly improve involution of the vascular plexus around each hair follicle (Figure 5). D/P MPs may thus provide an excellent biomaterial to immobilize, retain, and gradually release various GFs in PRP for induction of development of hair follicles.

We do not have evidence of D/P MPs alone on hair growth as control. Our preliminary results showed that D/P MPs alone had an effect of angiogenesis

in vivo using rabbit but lower than that of PRP&D/P MPs (data not shown). Therefore, it can not be excluded that D/P MPs alone may have some inherent stimulatory effects on hair growth. We plan to conduct a clinical study to evaluate D/P MPs alone for hair growth.

The results of this clinical study show a significant improvement in hair thickness after local injection of PRP&D/P MPs. PRP derived from the individual is autologous material, with few allergic side effects. In addition, because all components for PRP&D/P MPs, namely dalteparin, protamine, and PRP, are in clinical use, safety for clinical use appears likely.

## Conclusions

This clinical research was conducted using PRP&D/P MPs and PRP alone in 26 participants with thin hair, including 10 women. Hair growth and thickening after administration of PRP&D/P MPs and PRP were observed in all participants, but PRP&D/P MPs appeared to provide more-substantial changes than PRP alone. This method with PRP&D/P MPs is simpler and cheaper than conventional methods and has no side effects because of the use of autologous materials.

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