# Autologous Platelet-Rich Plasma: A Potential Therapeutic Tool for Promoting Hair Growth

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BACKGROUND Recently, autologous platelet-rich plasma (PRP) has attracted attention in various medical fields, including plastic and orthopedic surgery and dermatology, for its ability to promote wound healing. PRP has been tested during facelift and hair transplantation to reduce swelling and pain and to increase hair density.

OBJECTIVE To investigate the effects of PRP on hair growth using in vivo and in vitro models.

METHODS PRP was prepared using the double-spin method and applied to dermal papilla (DP) cells. The proliferative effect of activated PRP on DP cells was measured. To understand the mechanisms of activated PRP on hair growth, we evaluated signaling pathways. In an in vivo study, mice received subcutaneous injections of activated PRP, and their results were compared with control mice.

RESULTS Activated PRP increased the proliferation of DP cells and stimulated extracellular signal-regulated kinase (ERK) and Akt signaling. Fibroblast growth factor 7 (FGF-7) and beta-catenin, which are potent stimuli for hair growth, were upregulated in DP cells. The injection of mice with activated PRP induced faster telogen-to-anagen transition than was seen on control mice.

CONCLUSIONS Although few studies tested the effects of activated PRP on hair growth, this research provides support for possible clinical application of autologous PRP and its secretory factors for promotion of hair growth.

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Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. Although the optimal PRP platelet concentration is unclear, the current methods by which PRP is prepared involve reported 300% to 700% enrichment, with platelet concentrations consequently increasing to more than 1,000,000 platelets/μL.[1,2]

PRP has attracted attention in several medical fields because of its ability to promote wound healing. Generally, platelets were previously thought only to contribute to hemostasis, but they are now known to initiate wound healing by secreting various growth factors and cytokines. In this process, referred to as "activation," platelet alpha granules become activated and release numerous proteins, including platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and interleukin (IL)-1.[3,4]

PRP has also attracted attention in plastic surgery and dermatology because of its potential use during facial plastic surgery and aesthetic, skin-rejuvenating effects. Laboratory studies have shown that

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PRP enhances the proliferation of human dermal fibroblasts and human adipose-derived stem cells, whereas recent clinical reports describe the use of PRP as a scaffold for injectable soft tissue augmentation and a treatment for acne scars and nasolabial folds.[5–10] Another recent study evaluated the effects of treatment with platelet plasma growth factors during male pattern baldness surgery.[11] The authors observed a significant improvement in hair density and stimulation of growth when follicular units were pretreated with platelet plasma growth factors before their implantation. They hypothesized that growth factors released from platelets may act on stem cells in the bulge area of the follicles, stimulating the development of new follicles and promoting neovascularization.

In spite of these previous studies, the precise mechanism by which PRP promotes hair growth has not been properly studied. No experimental studies have attempted to define the specific effects of PRP on human hair follicles. The aim of this study was to determine the effects of PRP on human hair follicle growth in vitro and in vivo and to explore the possible mechanisms involved.

## Methods

### Cell Culture

Specimens of normal human scalp skin were obtained during dermatologic surgery in accordance with the ethical committee approval process of Chungnam National University Hospital. Dermal papilla (DP) cells were cultured according to a previously described method[12] with slight modifications. The isolated DP cells were transferred to Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide.

### Preparation of Activated PRP

PRP was prepared using a double-spin method. Briefly, 10 mL of blood obtained from healthy adult volunteers ($n$ = 10) who had provided informed consent was transferred to tubes containing acid-citrate-dextrose solution A anticoagulant (1:4 vol/vol mixture). The citrated blood was centrifuged at 660 $g$ for 7 minutes. Subsequently, the yellow plasma (containing the buffy coat comprising platelets and leukocytes) was aspirated using a micropipette and then centrifuged at 2,350 $g$ for 5 minutes, yielding a platelet-rich pellet and platelet-poor plasma (PPP). The platelet-rich pellet was resuspended in 1.0 mL of plasma and the resulting suspension used as PRP. A 1:1 mixture of 0.5 M calcium chloride and thrombin was prepared in advance for use as an activator. A 10:1 mixture of PRP and the activator was incubated for 10 minutes at room temperature, yielding activated PRP. The activated PRP was centrifuged at 16,600 $g$ for 15 minutes. The supernatant was stored at −20°C before use.

### Cell Proliferation Assay

The proliferation of DP cells was measured in a previously described [$^3$H]-thymidine incorporation assay.[13] DP cells were treated with 1 μCi of [$^3$H]-thymidine (Amersham Biosciences, Buckinghamshire, UK) and incubated for the indicated periods of time. Levels of radioactivity in cell lysates were measured using a liquid scintillation counter.

### Western Blotting

Cells were lysed in radioimmunoprecipitation assay buffer. Total protein samples were electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gels, transferred to nitrocellulose membranes, and incubated overnight at 4°C with primary antibodies. Blots were then incubated with peroxidase-conjugated secondary antibodies for 1 hour at room temperature, and visualized using an enhanced chemiluminescence reagent (Intron, Gyeonggi, Korea).

### Enzyme-Linked Immunosorbent Assay

Cells ($1 \times 10^5$) were grown to confluence in DMEM supplemented with 10% FBS. They were

serum starved for 24 hour and cultured for a further 2 days in fresh DMEM supplemented with 5% FBS, 5% activated PRP, or 10% activated PRP (3 mL/well). Levels of FGF-7 in the conditioned media were quantified using an enzyme-linked immunosorbent assay kit (cat # DKG00; R&D Systems Inc., Minneapolis, MN).

### Animal Study

The most widely used inbred strain, female C57BL/6 mice (Orient Bio. Inc., Gyeonggi, Korea) were used in an animal experiment because of their dark black hair, in which it is easy to observe hair growth. The Chungnam National University's Institutional Animal Care and Use Committees (CNU-COM-2007–017) approved the experiment, and the Guideline for the Care and Use of Laboratory Animals was observed. At age 7 weeks, when all hair follicles were in the telogen phase, the dorsum of each mouse was carefully shaved with an animal clipper. Mice were randomly assigned to one of three groups. The dorsal skin of each mouse was subcutaneously injected with 100 μL of phosphate buffered saline (PBS) for negative control, FBS for positive control, or activated PRP at 3-day intervals. Hair growth was monitored for 3 weeks, and injections were stopped before the mouse hair cycle reentered the anagen cycle.

### Statistical Analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). $P < .05$ was regarded to be statistically significant. Data are presented as means ± standard deviations.

## Results

### Concentration of PRP

Prepared using a double-spin method, PRP has a higher platelet concentration than PPP or whole blood. Mean platelet counts in whole blood, PRP, and PPP were $1.8 \times 10^5$, $1.6 \times 10^6$, and $6.3 \times 10^4$ cells/μL, respectively. The concentration of platelets in PRP was approximately 8.8 times as great as that in whole blood.

### PRP Induces the Proliferation of DP Cells

To determine whether PRP promotes the proliferation of human DP cells, we performed [$^3$H]-thymidine incorporation assay. As shown in Figure 1, the rate of proliferation on culture day 3 was higher in DP cells treated with activated PRP than in untreated control cells. Increasing the concentration of activated PRP did not seem to increase the proliferation of DP cells.

### PRP Increases the Phosphorylation of Extracellular Signal-Regulated Kinases and Akt and Bcl-2 Expression in DP Cells

We investigated the effects of activated PRP on levels of phosphorylated extracellular signal-regulated kinases (pERK) and phosphorylated Akt (pAkt) in cultured human DP cells. Because activation of ERK signaling is known to induce cell growth, and activation of PI 3-kinase/Akt signaling promotes cell survival and prevents apoptosis, it suggests that activated PRP elevates expression of

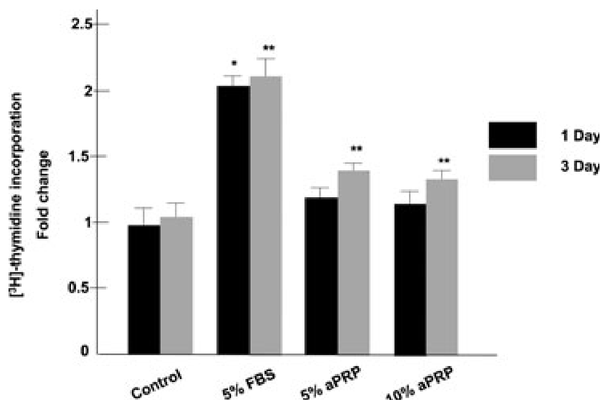


**Figure 1.** The effect of activated platelet-rich plasma (PRP) treatment on the proliferation of cultured human dermal papilla (DP) cells. After serum starvation for 24 hours, human DP cells were cultured with 5% fetal bovine serum, 5% activated PRP, or 10% activated PRP in addition to growth medium (Dulbecco's modified Eagle medium [DMEM]). Activated PRP enhanced the proliferation of cultured human DP cells. *$P < .05$ vs control (nonsupplemented DMEM) on day 1, **$P < .05$ vs control on day 3.

pAkt and pERK in DP cells and that activated PRP has a potential role in inducing hair growth in vivo. pERK and pAkt levels were quantified using Western blotting after incubation for PI3 kinase for 48 hours with nonsupplemented DMEM (control) or DMEM supplemented with 5% FBS (positive control), 5% activated PRP, or 10% activated PRP. pERK and pAkt levels were significantly higher in activated PRP-treated human DP cells than in control and 5% FBS-treated cells (Figure 2A). The effects of activated PRP were dose dependent.

Because mitogen-activated protein kinase signaling contributes to cell survival by modulating the expression of apoptotic molecules, we also investigated the effects of activated PRP on the expression of the anti-apoptotic protein Bcl-2. Bcl-2 protein levels were higher in human DP cells treated with activated PRP than in control and 5% FBS-treated cells (Figure 2B).

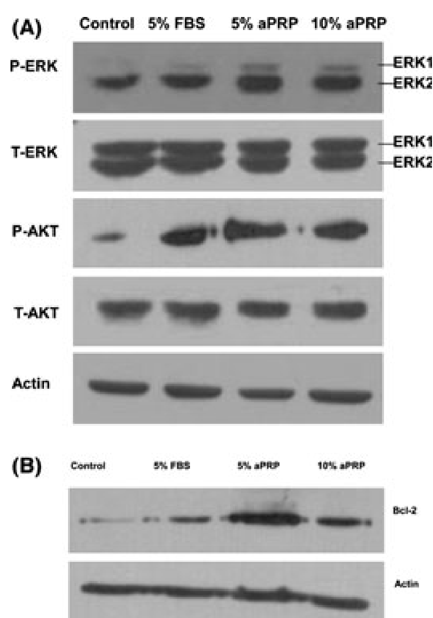


**Figure 2.** The effect of activated platelet-rich plasma (PRP) treatment on phosphorylated extracellular signal-regulated kinase (p-ERK), p-Akt, and Bcl-2 levels in cultured human dermal papilla (DP) cells. (A) Activated PRP increased the phosphorylation of ERK and Akt in cultured human DP cells. (B) Activated PRP treatment significantly increased the expression of Bcl-2 expression.

### *PRP Increases Beta-Catenin Activity and FGF-7 Expression in DP Cells*

Because beta (β)-catenin signaling plays important roles in hair follicle development and the hair growth cycle, we measured the transcriptional activity and protein level of β-catenin in DP cells treated with activated PRP. Treatment with activated PRP approximately doubled transcriptional activity of β-catenin, confirming that the exogenously expressed β-catenin was active (Figure 3A). Western blotting analysis showed β-catenin levels to be higher in cells treated with activated PRP than in control cells (Figure 3B).

We also investigated FGF-7, which is located in the DP cells and prolongs the anagen phase of the hair cycle and delays progression into the catagen phase.[14] Protein levels of FGF-7 were higher in DP cells treated with activated PRP (Figure 3C), and the level of secreted FGF-7 was significantly greater in cultured medium treated with activated PRP after 1 and 2 days (Figure 3D).

### *PRP Stimulated Hair Growth in an Animal Model*

To evaluate the effects of activated PRP on hair growth in vivo, we injected PBS (control), FBS (positive control), or activated PRP subcutaneously at two points on the lateral skin of C57BL/6 mice. Diffuse darkening of the dorsal skin was observed after injection of PRP once every 3 days for 2 weeks. Mice injected with activated PRP for 3 weeks exhibited near-complete hair regrowth, whereas PBS- and FBS-injected mice did not (Figure 4).

## Discussion

There has been much effort put into identifying medications and medical procedures that promote hair growth, because many people world-wide have thinning or loss of hair. Although finasteride has been the mainstay treatment for advanced androgenetic alopecia, there are no indicated treatments for women experiencing postpartum, perimenopausal,

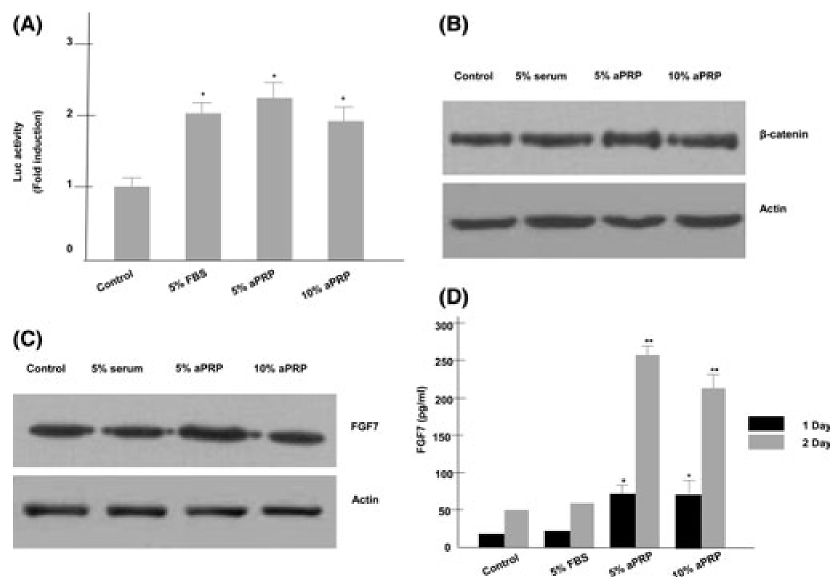


**Figure 3**. The effect of activated platelet-rich plasma (PRP) on the expression of β-catenin and secretion of fibroblast growth factor (FGF)-7 in cultured human dermal papilla (DP) cells. (A) The transcriptional activity of β-catenin was assayed with luciferase activity. Activated PRP increased luciferase activity in cultured human DP cells. (B) Activated PRP increased the expression of β-catenin protein in cultured human DP cells. (C) Western blot analysis of FGF-7 protein in human DP cells. FGF-7 expression was greater in activated PRP-treated human DP cells. (D) The level of secreted FGF-7 in culture medium treated with fetal bovine serum or activated PRP were measured using enzyme-linked immunosorbent assay. FGF-7 was greater in activated PRP-treated culture medium after 1 and 2 days.

or menopausal hair loss and for younger men experiencing the early stages of androgenetic alopecia.

PRP has found application in many surgical fields because of its ability to stimulate wound healing and minimize bleeding during surgery. PRP has recently attracted the attention of plastic surgeons and dermatologists because of the ability of growth factors contained in the alpha granules of platelets, including PDGF, TGF, VEGF, and IGF, to stimulate human dermal fibroblasts, improve wrinkling, and rejuvenate the skin.[6,9,10,15] PRP has also een shown to increase the yield of transplanted follicular units when applied during male pattern baldness surgery,[11] although the effects of PRP on hair growth and the mechanisms by which PRP stimulates hair follicles have not been thoroughly investigated.

We prepared PRP using a double-spin method, in which blood cell layers were manually separated. The activation of platelets through coagulation triggers the secretion of various growth factors, which produce mitogenic effects in various cell types.[16,17] The proliferation of osteoblasts and alveolar bone cells[17] treated with 1% to 5% PRP was found to be higher than that of cells treated with higher concentrations of PRP. Similarly, we found that activated PRP increased the proliferation of human DP cells when applied at concentrations of 5% and 10% and that the proliferation of DP cells treated with 5% activated PRP tended to be slightly higher than that of cells treated with 10% activated PRP, although the difference was not statistically significant. Activated PRP also increased Bcl-2 protein levels. A member of the Bcl-2 family of apoptosis regulators, Bcl-2 suppresses apoptosis in a variety of cell systems. Continuous expression of Bcl-2 in DP cells during the hair cycle suggests that DP cells may normally be protected from apoptosis.[18] Taken together, our data suggest that activated PRP promotes the proliferation of DP cells and protects them from apoptosis.

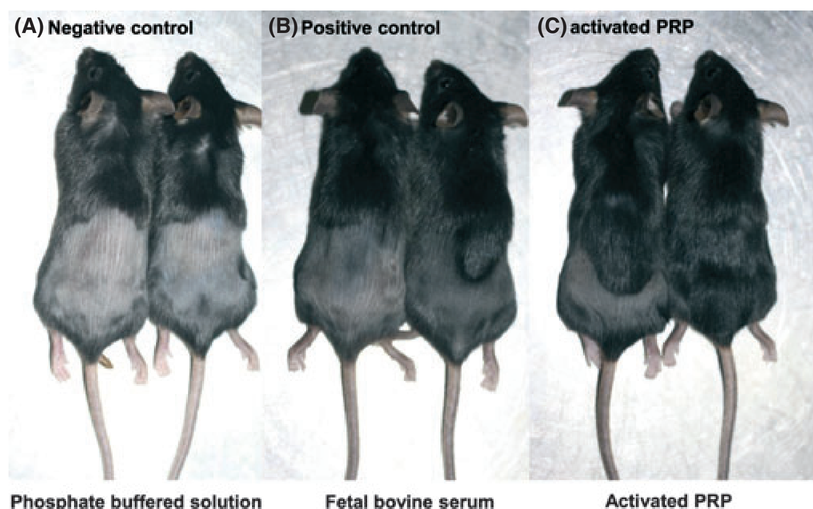


**Figure 4**. The effect of activated platelet-rich plasma (PRP) on the hair growth of C57BL/6 mice. C57BL/6 mice were shaved when 7 weeks old and then (A) phosphate buffered saline (PBS; control), (B) fetal bovine serum (FBS; positive control) or (C) activated PRP was subcutaneously injected at 3-day intervals. Near-complete hair regrowth was observed in mice injected with activated PRP for 3 weeks.

To determine how PRP stimulates hair growth, we measured levels of phosphorylated ERK and Akt in untreated DP cells and cells treated with activated PRP or 5% FBS. Although ERK signaling contributes to the regulation of cell growth, Akt is known to be an important player in cell survival and has anti-apoptotic effects in many cell types.[19] Our results suggest that activated PRP increases cell growth and prolongs the survival of hair follicles by activating ERK and Akt signaling, respectively.

Major molecular pathways known to be involved in the development of skin appendages relevant to hair growth—the β-catenin/lymphoid enhancer-binding factor-1 and FGF-7 signaling pathways—were also investigated. β-catenin is strongly expressed in the outmost outer root sheath layer in the bulge region of the human anagen hair follicle and is known to play a dual role in hair follicles, contributing not only to the formation of hair placodes, but also to the differentiation of stem cells into hair follicle cells and other skin cells in adults.[20] The observed upregulation of β-catenin activity in activated PRP-treated DP cells reinforces the idea that activated PRP stimulates hair growth by inducing the differentiation of stem cells into hair follicle cells. The marked increase in the expression of FGF-7 in DP cells treated with activated PRP further suggests that activated PRP may prolong the anagen phase of the hair cycle and stimulate hair growth.

In support of the in vitro data, the injection of activated PRP into the skin of mice once every 3 days for 3 weeks greatly stimulated hair growth. According to the results, PRP increased hair growth and hair follicle survival owing to its promotion of cell proliferation and its anti-apoptotic properties. Thus, PRP can potentially prolong the anagen phase of the hair growth cycle. PRP can be easily obtained from a small amount of the patient's blood and can be added directly to the follicular units by dipping the follicles in PRP before implantation during hair transplantation. PRP may also be used after hair transplantation to minimize bleeding and stimulate wound healing. PRP appears to stimulate the survival of implanted hair follicles and hair growth, although the best method of delivering activated PRP to human scalp skin and the optimal treatment interval must be established for PRP to be a useful therapy in patients.

We have demonstrated that activated PRP stimulates the proliferation of human DP cells, increases

the survival of hair follicle cells through its anti-apoptotic effects on DP cells, and may stimulate hair growth by prolonging the anagen phase of the hair cycle. Considering the limited evidence as to its clinical efficacy and safety, further studies are required to investigate the mechanism of action and safety of autologous blood–derived PRP before it may be applied clinically.

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