Hair loss is a common but upsetting complaint for both men and women. To help combat the problem, Vivimed has developed Vividine, an active ingredient that helps regenerate hair growth and prevent further hair loss. Scientists believe that Vividine works by increasing the blood flow to the hair follicles, bringing a healthy dose of rich nutrients to the scalp, which revive the hair follicles and prevent further loss and thinning.

A typical head of human hair will number some 100,000-150,000 hairs, and each individual hair grows and falls out through three phases of the hair growth cycle:
1. **Anagen**: the active growth phase, hair grows on average 1cm/month.
2. **Catagen** phase signalling the end of the growth phase. Metabolic process slows.
3. **The telogen** following the catagen is a phase when hair papilla becomes smaller and hair follicles recede, so that the dead hair shafts are shed.

This life cycle of hair average 3—5 years and can vary depending on various conditions, including nutrition, medical history, heredity, physical constitution, hormone secretion and aging.

**Hair Loss**
Hair loss is the medical description of the loss of hair from the head or body, sometimes to the extent of baldness. Unlike the common cosmetic depilation of body hair, alopecia tends to be involuntary and unwelcome, e.g., androgenic alopecia. In some cases, alopecia is an indication of an underlying medical concern, such as iron deficiency.

When hair loss occurs in only one localised area, it is known as alopecia areata. Alopecia universalis is when complete hair loss on the body occurs, similar to how hair loss associated with chemotherapy sometimes affects the entire body.

**Hair Growth**
Hairs grow in cycles which are not synchronized in human beings; each hair enters phases of the growth cycle at a different time. There are three phases of the hair growth cycle: anagen, catagen and telogen.

**Anagen** is the phase of active hair growth - approximately 90% of all hairs will be in anagen phase, typically lasting from 2 to 6 years, depending on skin region.

After anagen is completed, the hair enters catagen; during this short phase (2 - 3 weeks) the matrix cells gradually stop dividing and begin to keratinize.

When full keratinization is achieved, the hair enters the last phase of the cycle, telogen.
Vividine

Up to 10% of the hair will be in telogen phase at any one time. It is usual to shed about 100 hairs per day in telogen phase. During this phase (3 - 4 months) keratinized hair falls out, and a new matrix is gradually formed from stem cells in basal layer of outer epithelial root sheath bulge. A new hair starts to grow and the follicle returns to anagen phase.

Factors Influencing Hair Growth

Stem cells of the hair follicle are gathered in the basal layer of the outer root sheath bulge. It is from these cells that matrix cells are formed. Growth and differentiation of the matrix cells are under the influence of substances produced by cells of the dermal papilla. On the other hand, the secretory activity of the dermal papilla is controlled either by substances produced in cells of the spinous layer of the outer root sheath or by hormones.

Cells of the spinous layer produce peptides greater than 3000 daltons which increase the number of papilla cell mitoses two to five times. It was recently discovered that basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) potentiate the growth of dermal papilla cells. It is proposed that these proteins increase the synthesis of stromelysin (an enzyme, matrix metalloproteinase) which acts on the papilla cells and accelerates their growth.

Another cytokine, transforming growth factor beta (TGF-β), inhibits mitogen-induced dermal papilla cell proliferation.

On the other hand, dermal papilla cells produce numerous cytokines which influence proliferation of hair matrix cells. Some of them are stimulators, and some inhibitors. Interleukin 1-α (IL-1α) inhibits growth of hair and follicle, but only after 2-4 days of latency. The increase of IL-1α concentration in extracellular fluid during inflammation could be one of the reasons for alopecia following certain infectious diseases.

Apart from IL-1alpha, both fibroblast growth factor (FGF) and epidermal growth factor (EGF) inhibit growth of the hair and hair follicle. Fibroblast growth factor type 5 (FGF5) is an especially potent inhibitor.

Receptors for these ligands were found by immunohistochemical methods on papilla cells, matrix cells and stem cells in the bulge region of the hair follicle.

Another cytokine produced by cells of the dermal papilla, keratinocyte growth factor (KGF), induces extensive hair growth in murine models of alopecia. Receptors for KGF were found on keratinocytes in the basal epidermis and throughout developing hair follicles of rat embryos and neonates.

Insulin-like growth factor I (IGF-I) accelerates, in a concentration-dependent manner, growth of hair and hair follicles. The actions of IGF-I are modulated by proteins produced in dermal papilla cells which bind IGF (insulin-like growth factor-binding proteins: IGFBPs); the exact mechanism of modulation has not yet been resolved. However, it has been shown that IGFBP-3 (which is the most abundant IGFBP type in dermal papilla cells) forms a complex with free IGF-I to reduce the concentration of IGF-I available for stimulation of hair elongation and maintenance of the anagen phase.

Retinoids and glucocorticoids stimulate production of IGFBP-3 in dermal papilla cells. Insulin itself has the same effect as IGF-I; it has been observed that body hair in patients with hyperinsulinism has a male distribution pattern. On the other hand, growth hormone (somatotropin) has no direct influence on follicle and hair growth.

Mechanism

- Vividine, *Pyrrolidinyl dianinopirimidine oxide*, smoothly supplies nutrients necessary for hair growth through the vasodilatation of blood vessels around hair follicles to promote the transition from the telogen phase to the anagen phase of the hair cycle and to maintain the anagen phase for a long period of time.

- Vividine shows a potassium channel opening effect through antagonistic action against a potassium channel opening-inhibitory drug, and the effect of promoting the proliferation of dermal papillae cells, and has the effect of stimulating the transition from the telogen phase to the anagen phase of the hair cycle in mice having the telogen phase.

- Vividine is an active ingredient, and thus inhibits general male-pattern or female-pattern hair loss and promotes hair growth.

- Vividine has the effect of promoting the proliferation of dermal papillae cells.
Vividine

**Applications**
To regenerate hair growth and prevent hair loss in both men and women.

- For the treatment of hair loss, Pyrrolidinyl Diaminopyrimidine ('PDP') Oxide is applied as a topical solution that is generally between 1% to 5% active Vividine in propylene glycol.
- The propylene glycol ensures that the applied active ingredient is evenly spread across the affected area and easily absorbed through the skin. As of early 2001, the 5% solution is only approved by the FDA for use on men.
- **Vividine** is not a cure for baldness, but it has been shown to retard recent hair loss and to stimulate new hair growth, particularly in the crown of the scalp in certain, usually younger, men.
- Applied to the scalp VIVIDINE is used to stimulate hair growth in adult men and women with a certain type of hair loss and hair thinning.
- It is believed to work by helping to increase blood flow to the hair follicles by dilating blood vessels. Results usually take place after several months of daily use. It only works as long as it is used. In the U.S., this treatment is available without prescription.
- PDP oxide is clinically proven to regrow hair and stop thinning hair. **Vividine** regrows more hair than most hair loss treatments do and it can produce results within 6-8 weeks.
- **Vividine** is used to stimulate hair growth in areas of the scalp that have stopped growing hair.
- It is used to stimulate hair growth and to slow balding. It is most effective for people under 40 years of age.

**Incorporation Level**
PDP oxide is preferably used in amounts of 0.001-5.0 wt% depending on the formulation and desired application method.

**Formulation types**
Hair tonic—Scalp treatment—Hair shampoo
Hair cream—General ointment—Skin lotion
Milk lotion—Eye cream—Nourishing cream
Massage cream—Cleansing cream
Cleansing foam or powder

**Clinical trials**
1. Test of hair growth effect in mice
   The dorsal hair of 47-53-day-old mice (C57BL/6) was removed, and among the mice, mice having a clean back portion were selected. The selected mice were divided into 18 groups each consisting of 8 animals. The mouse groups were applied daily with 150 μH of each of the following substances for 21 days to test the hair growth effects of the substances:
   a) a negative control group consisting of water/ethanol/1,3-butyleneglycol (5/3/2)
   b) a composition obtained by dissolving PDP oxide in the negative control vehicle to a concentration of 0.5 wt%
   c) and a composition obtained by dissolving each of the following substances in said PDP oxide-containing composition to a concentration of 1 wt%: Thujae semen, vitamin B5 derivatives (panthenol derivatives), Swertia extract (Swertiall), Coicis semen, Glycyrrhiza extracts (glycyrrhizin and glycyrrhetic acid), nicotinamide (niacinamide), vitamin E derivatives (tocopherol acetate, etc.), adenosine, glyceryl pentadecanoate (PDG), 6-benzyl aminopurine, eugenol, saw palmetto, dialkylmonoamine derivatives, isoflavone, hinokitiol and benzylnicotinate.

   After 21 days, the mice were photographed to observe the growth of hair in the mice, and the weight of newly grown in the test groups was measured, and compared with that in the negative control group.

<table>
<thead>
<tr>
<th>Hair Wt (mg)</th>
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<tbody>
<tr>
<td>Negative control group</td>
</tr>
<tr>
<td>PDP Oxide group</td>
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</table>

The PDP oxide according to the present invention showed an increase of about 29 mg in hair weight compared to the group applied with the negative control group (water/ethanol/1,3-butyleneglycol). Also, it could be observed that the group applied with the PDP oxide together with each of the 16 kinds of components, which can be selectively used in the present invention, showed increased hair growth compared to the group treated with PDP oxide alone. The PDP oxide acted on the mice in the telogen phase to stimulate the transition to the anagen phase of the hair cycle, thus promoting hair growth in the mice.
2. Potassium channel opening effect
PDP oxide 5% bovine fetal serum and 100 IU-penicillin were added to a DMEM medium containing phenol red, and the medium was added onto a 24-well multi-well culture plate.

A mouse-derived fibroblast NIH3T3 cell line was dispensed onto the multiwall culture plate at a density of 10,000 cells/well. Potassium channel antagonist tolbutamide was added to the medium at a final concentration of 2.5 nM, and a 100-fold dilution of PDP oxide was added to the medium at concentrations of 1 μM, 50 μM and 100 μM, and the medium was incubated at 37 °C for 3 days.

After the incubation, a solution of 0.2% MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) was added to the microtiter plate in an amount of 200 μl per well, and the culture medium was further incubated at 37 °C for 3 days.

Then, the produced formazane was dissolved in DMSO (dimethylsulfoxide). The absorbance of the dissolved formazane was measured at 570 nm using a microplate reader. The PDP oxide suppressed the potassium channel-antagonistic action of the tolbutamide at each concentration to promote the proliferation of NIH3T3.

PDP oxide did not show the hair growth promoting effect resulting from the potassium channel opening effect as high as the level of the control group, but showed a high growth-promoting effect compared to the negative control group treated only with tolbutamide.

3. Test of effect on promotion of dermal papillae cells
Rat-derived dermal papillae cells cultured in DMEM (Dulbecco’s Modified Eagle's Media) containing 2% bovine fetal serum were dispensed onto a 96-well multi-well culture plate at a density of 1,000 cells/well, and 10 μg/ml of positive control group and PDP oxide was the culture medium.

Also, each of 10 μg/ml of Thujae semen, vitamin B5 derivatives (panthenol derivatives), Swertia extract (Swertiall), Coicis semen, Glycyrrhiza extracts (glycyrrhizin and glycyrrhetic acid), nicotinamide (niacinamide), vitamin E derivatives (tocopherol acetate, etc.), adenosine, glyceryl pentadecanoate (PGD), 6-benzylaminopurine, eugenol, saw palmetto, dialkyldiaminoamine derivatives, isoflavone, hinokitiol and benzynicotinate was added to the culture medium together with said PDP oxide.

Then, the culture media were incubated at a temperature of 37 °C for 48 hours.

After the incubation, a solution of 0.2% MTT [3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide] was added to the micro titer plate at a density of 50 μl per well, and the culture media were further incubated at 37 °C for 4 hours.

The produced formazane was dissolved in DMSO (dimethylsulfoxide). The absorbance of the dissolved formazane was measured at 570 nm using a microplate reader. The measurement results for the test groups were compared with a control group treated only with the DMSO solution to measure a difference in absorbance between the test groups containing the PDP oxide.
Vividine

PDP oxide showed a papillae cell proliferation capability equal to that of control, and the papillae cell proliferation capability of the group treated with the PDP oxide together with each of the sixteen components was higher than that of the group treated with the PDP oxide alone.

The papillae cell proliferation capability of the PDP oxide according to the present invention was measured at varying concentrations of 0.1, 1 and 10 ppm in the same manner as described above, and the measurement results were compared with those of a control group (CTL) treated only with the DMSO solution to determine a relative difference in absorbance.

The measurement results show that the papillae cell proliferation capability of the PDP oxide was increased in a dose-dependent manner.

<table>
<thead>
<tr>
<th>Appearance:</th>
<th>Pale white crystalline powder</th>
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</thead>
<tbody>
<tr>
<td>Purity by HPLC:</td>
<td>≥ 99.0 %</td>
</tr>
<tr>
<td>Moisture content:</td>
<td>≤ 0.5%</td>
</tr>
<tr>
<td>Heavy metal content:</td>
<td>≤ 20 ppm</td>
</tr>
<tr>
<td>pH:</td>
<td>7.5 - 9.5 (3% aq. sol’n)</td>
</tr>
</tbody>
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References
Vividine

Product Specification

Appearance: Pale white crystalline powder

Purity by HPLC: ≥ 99.0 %

Moisture content: ≤ 0.5%

Heavy metal content: ≤ 20 ppm

pH: 7.5 - 9.5 (3% aq. sol’n)

Solubility Data: Soluble in butylene glycol, propylene glycol and binary solvents such as water/ethanol, glycol/castor oil mix. Not soluble in water.

Known incompatibilities: Avoid contact with strong oxidising agents

Application information: Dissolve up in suitable solvent before adding to formulation. Usage level 0.1—5% depending on delivery system.

Packaging: Standard 10kg fibre kegs and HDPE drums

Storage recommendations: Shelf life 4 years when stored in original sealed container - store in cool, dry environment away from direct sunlight and below 25ºC.

Vivimed’s range of hair care products

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vividine</td>
<td>Hair growth stimulant</td>
</tr>
<tr>
<td>Dantuff-Z</td>
<td>Zinc pyrithione anti-dandruff active</td>
</tr>
<tr>
<td>Dantuff-C</td>
<td>Climbazole anti-dandruff active</td>
</tr>
<tr>
<td>Vipirox</td>
<td>Piroctone olamine anti-dandruff agent</td>
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<tr>
<td>Tru Aloe</td>
<td>Emollient, moisturiser</td>
</tr>
<tr>
<td>Allantoin</td>
<td>Anti-inflammatory, moisturiser</td>
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<tr>
<td>Avis, Etone, Octyne-b, Ben-3, Ben-4, Vivsonic</td>
<td>UV-filters</td>
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<tr>
<td>Vivilide, Vivmax, Viv-20, Cosvat</td>
<td>Anti-microbials &amp; preservatives</td>
</tr>
<tr>
<td>Co-Guar</td>
<td>Thickeners and conditioners</td>
</tr>
</tbody>
</table>

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