

# **Evaluation of the hair growth and retention activity of two solutions on human hair explants**

**Study Directed by Dr E. Lati of Laboratoire Bio-EC, Centre de Recherches Biologiques  
et d'Experimentations Cutanees, on behalf of Pangaea Laboratories Ltd.**

# Evaluation of the hair growth and retention activity of two solutions on human hair explants

*Independent Laboratory Evaluation and Experimental Report*

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**Quality Manager:** M. M. Daniel

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## Study Schedule

**Commence:** 25.01.2012  
**Complete:** 17.02.2012  
**Approved By:** Technical Manager  
M. C. Merle

**Partners:** None  
**Subcontractors:** None

## Abstract

Hair loss is often caused by abnormal hair follicle cycling and changes in hair follicle morphology. The molecules and biochemical pathways that control normal hair follicle formation, hair growth, and hair loss are beginning to be understood. Despite advances in hair research, current treatments on the market such as minoxidil and finasteride have limitations. Both solutions work very slowly, and finasteride particularly has some negative side effects therefore patient compliance for these treatments is low. An alternative or complementary treatment to topical minoxidil solution is highly desirable. Vascular Endothelial Growth Factor (VEGF), a cytokine naturally produced in the body has been characterised and identified as a key mediator of hair growth. With the aid of recombinant DNA technology, safe, identical copies of VEGF protein can be biosynthesised and used in treatments.

The objective of this study was to assess and evaluate the efficacy of Nanogen VEGF, or “Hair Growth Factor” and 5% minoxidil solution alone and in combination with each other. An *ex vivo* experimental model utilising human hair was designed to compare the effects of these two treatments.

Nanogen Hair Growth Factor outperformed minoxidil by as much as 237% and showed a complementary effect on minoxidil treatment when used in combination. All results were statistically significant.

## Introduction

Throughout the history of medicine, a tremendous amount of time has been dedicated to discovering external chemical molecules or elements, and applying them to the body to induce a physiological response.

It is only very recently that advances in molecular biology, and particularly proteomics, have described the mechanism of intercellular signals in great depth. We now know that many cellular functions, from mitosis and differentiation right through to apoptosis, are controlled by an array of biological molecules (proteins) known as cytokines. They can control all aspects of cellular proliferation and differentiation. Tissues in the body communicate constantly through a flurry of these signals.

Their existence is not a surprise to the scientific community, however the intensity and breadth of their effects was not fully understood and appreciated until now. The effect of cytokines or “growth factors” as they are commonly known is so significant that they would have great utility if they could be characterised, isolated and applied correctly as therapeutic agents.

In the field of hair research, VEGF (Vascular Endothelial Growth Factor) has been identified as a key mediator of hair growth (Figure 1). It can influence all stages of hair growth, from cell proliferation and migration through to angiogenesis and preventing apoptosis. It was hypothesised that cytokines and particularly VEGF can enhance hair growth and increase survival, reducing hair loss. If this were the case, VEGF could be used as an alternative to well known treatments such as minoxidil. It was also hypothesised that VEGF can improve response to minoxidil treatment if used in combination.



**Figure 1** Tertiary structure (3D) of Vascular Endothelial Growth Factor

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## Experimental Design & Methods

To test these hypotheses, an experiment was designed to compare the effects of Nanogen VEGF with 5% minoxidil, via an *ex vivo* experimental model utilising human hair. The objectives of this study were to measure and compare:

- Hair growth rates after topical application of Nanogen Hair Growth Factors and minoxidil.
- Hair follicle survival after topical application of Nanogen Hair Growth Factors and minoxidil.
- The effects of combination therapy with Nanogen Hair Growth Factors and minoxidil.

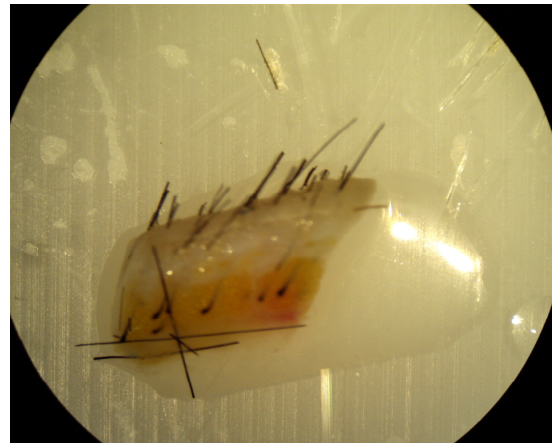
Bio-EC - an independent clinical testing laboratory based in France, was instructed to conduct a study investigating Nanogen Hair Growth Factors, using minoxidil as a positive control. The study utilised impartial measuring techniques, and was blind as the containers of growth factor and minoxidil were labelled anonymously.

## Products tested

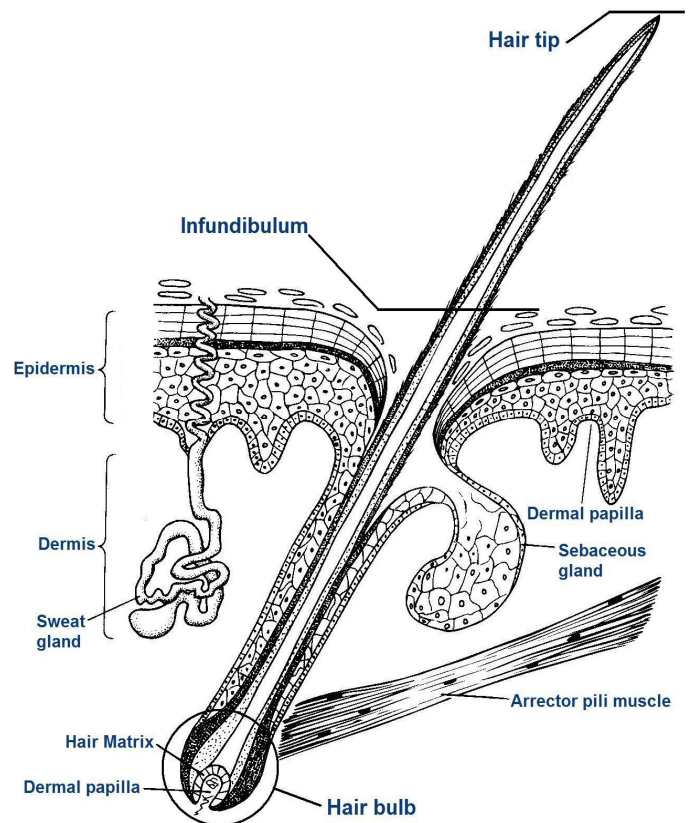
Sample Label	Identity	Visual Aspect	Quantity
A	Minoxidil	Transparent liquid	10ml
B	Nanogen VEGF	Transparent liquid	10ml
C	Nanogen VEGF + Minoxidil	Transparent liquid	10ml

## Preparation

A scalp plasty from a human donor was micro-dissected to isolated 80 individual hairs and their bulbs. Each hair was cut approximately 1mm above the infundibulum. (Figure 2.1, 2.2).



**Figure 2.2** Micro-dissection of a hair plasty.



**Figure 2.1** Anatomy of human hair

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## Screening

The hairs were then placed in 48-well plates and kept in classical survival cell culture conditions (Philpott medium, at 37°C in a 5% CO<sub>2</sub> atmosphere). The hairs were re-measured on day -4 (4 days post micro-dissection). Those demonstrating a healthy total growth of 54 to 1641 µm were selected, randomised and distributed into different batches of 7 hairs for product testing.

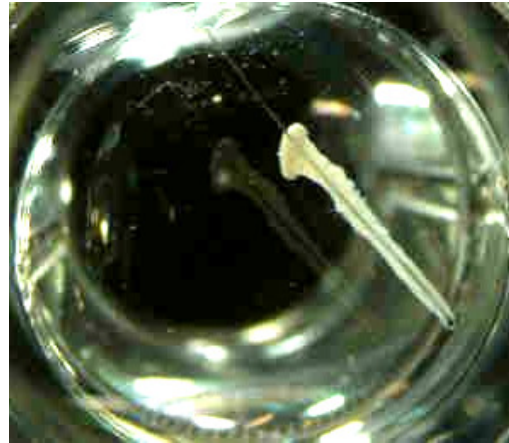
Treatment Hair Batch	Mean growth from D-4 to D0
Minoxidil	1063 µm
Nanogen VEGF	999 µm
Nanogen VEGF + Minoxidil	1172 µm

## Treatment & Sampling

After screening, the culture medium was renewed on day 0, 2, 4, 7 and 8. New treatment solution was also added to every well at each time point.

Treatment solution	Volume added to culture
Minoxidil 5%	15 µl
Nanogen VEGF	15 µl
Nanogen VEGF + Minoxidil	15 µl + 15 µl

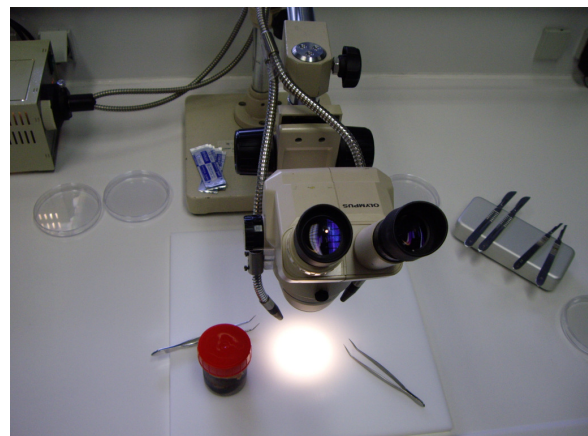
Hair samples were taken and measured at day 0, 4, 8 and 11. The growth of each hair was followed individually.



**Figure 2.3** Individual hair plasty in culture medium.

## Measurement

The length of each individual hair was measured from the bulb to the hair tip with the use of Olympus CellP software package. (Figure 2.4).



**Figure 2.4** Micro-dissection and micro-photography equipment.

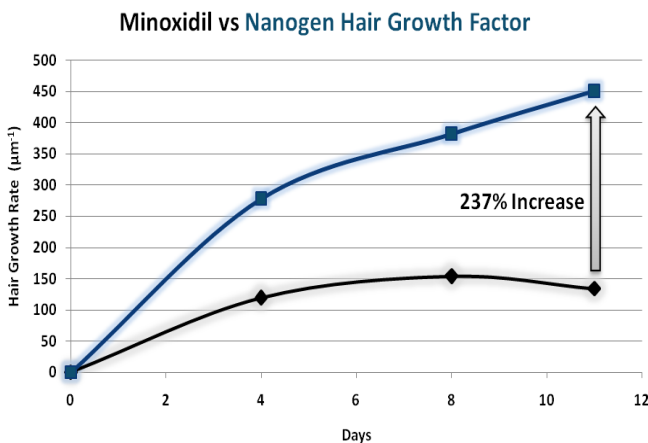
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### Results

When compared to minoxidil, Nanogen Hair Growth Factors are significantly more effective at stimulating hair growth. Up to 237% faster growth rate was shown in the growth factor group compared to the minoxidil positive control (Figure 3.1). A Student's t-test found a statistically significant difference between the two groups of treatment (p-value = 0.04).

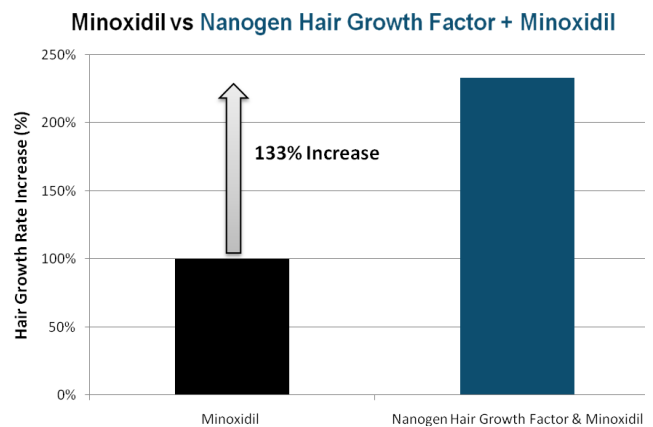
At the end of the hair cycle, as before hair falls out, necrosis (death) of some cells in the bulb region can occur. This is responsible for the small reduction in hair length. Treatment with Nanogen Hair Growth Factors allowed the hairs to continue growing at a healthy rate. In contrast, with minoxidil monotherapy, hair length begins to decline from day 9, indicating necrosis prior to the onset of hair loss. This shows that Nanogen Hair Growth Factors prolonged hair survival more effectively than minoxidil.



**Figure 3.1** Graph to compare average hair growth rate over time in minoxidil against Nanogen Hair Growth Factor.

After these results, the test was repeated to investigate the combination of Nanogen Hair Growth Factors with minoxidil, in comparison to minoxidil alone. 133% higher hair growth rate was observed with the combination treatment than with minoxidil treatment alone (Figure 3.2). A Student's t-test found a statistically significant difference in hair growth rate between the two groups of

treatment (p-value = 0.007). The complementary effect of Nanogen Hair Growth Factors on minoxidil treatment is clearly evident. However, the combination did not increase survival as expected. There is no theoretical basis for the two treatments to interact; therefore this may be due to the smaller sample number in the second test, or perhaps the increased dilution of Philpott medium caused by adding both treatment solutions.



**Figure 3.2** Graph to compare average hair growth rate over time in minoxidil against a combination of minoxidil and Nanogen Hair Growth Factor.

### Conclusion

It can be concluded that:

- Nanogen Hair Growth Factor treatment can significantly increase hair growth rate, up to 237% more than minoxidil monotherapy.
- Nanogen Hair Growth Factor treatment prolongs hair survival, and therefore can prevent or reduce hair loss.
- Nanogen Hair Growth Factors in combination with minoxidil can significantly increase hair growth rate by up to 133% more than minoxidil monotherapy.

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## QUALITY CONTROL STATEMENT

This study was conducted in compliance with Good Laboratory Practices (Arrêté du 10 Août 2004), as well as in compliance with the validated procedures and SOP of Laboratoire BIO-EC.

The audits performed ensure that all the steps of the study are controlled.

The dates and steps inspected during the various audits are presented in the table below:

<b>Audit type</b>	<b>Date</b>	<b>Step controlled</b>	<b>Dates of report to Study Director</b>	<b>Dates of report to the Director</b>
Externe	07/2006	Laboratory histology	27/07/2006	27/07/2006
Externe	22/06/2010	Laboratory histology	22/06/2010	22/06/2010
Externe	24/08/2010	Laboratory histology	24/08/2010	24/08/2010

This report was reviewed by the Quality Manager and confirms that the methods, standard operating procedures and observations are faithfully and completely described, and that the results reported reflect with accuracy the raw data of the study.

Quality Manager  
**M. Daniel**

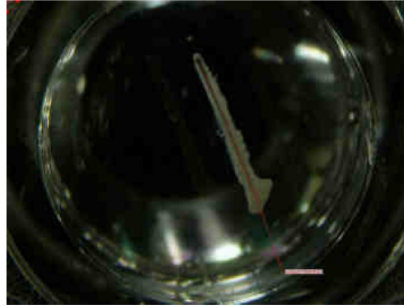


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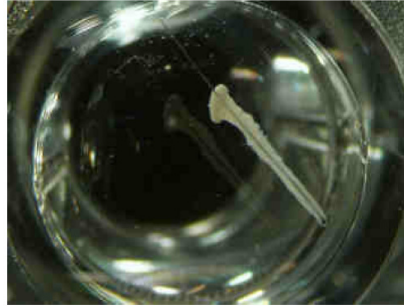
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**Appendix:** Selected microscope images of hair biopsies in wells at various stages.

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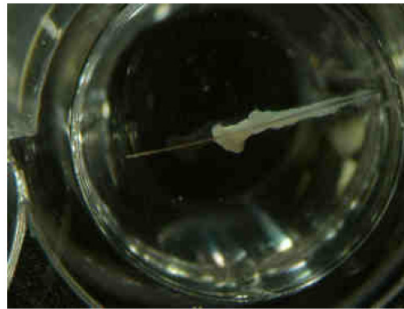
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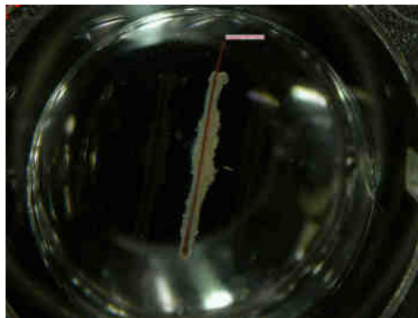
J8\_27



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Record Name: J0\_28



Record Name: J4\_28



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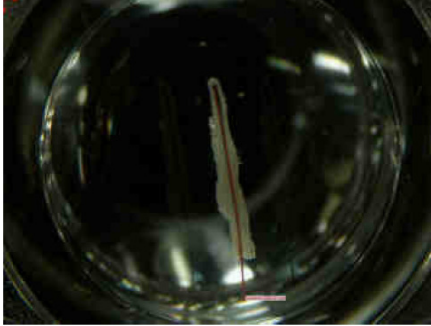
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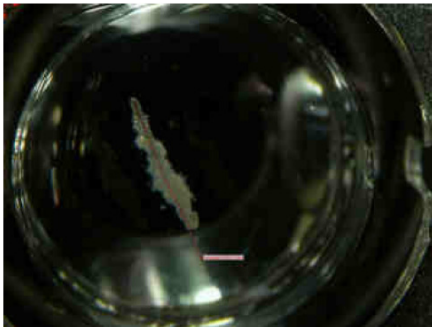
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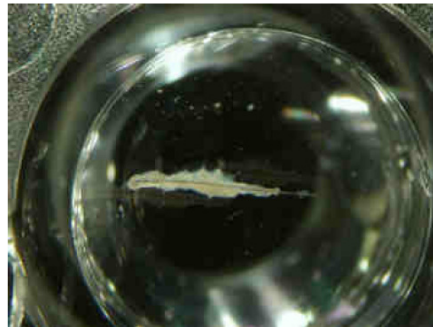
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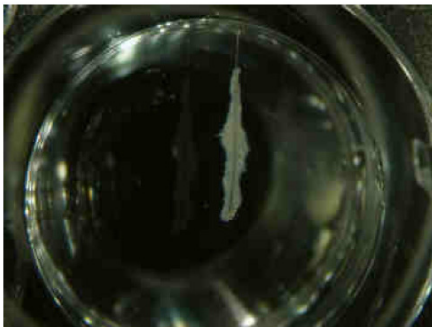
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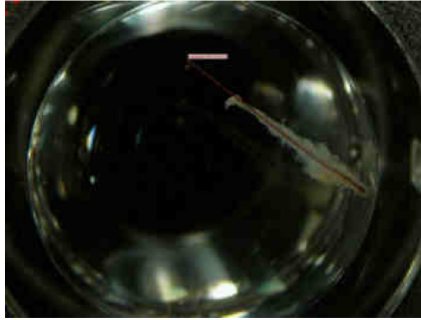
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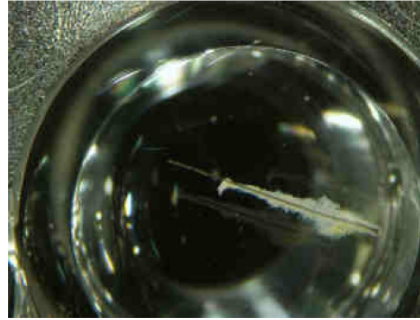
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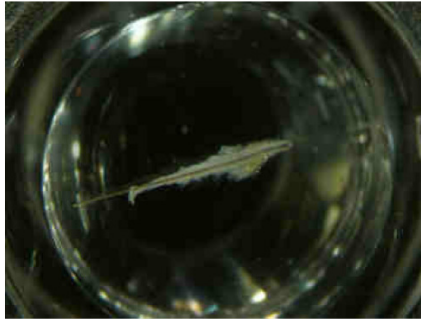
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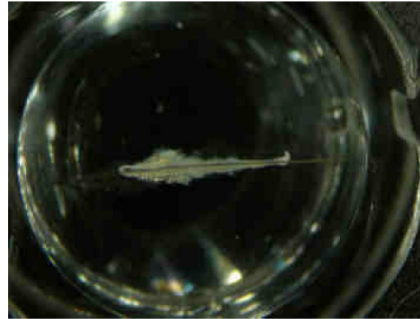
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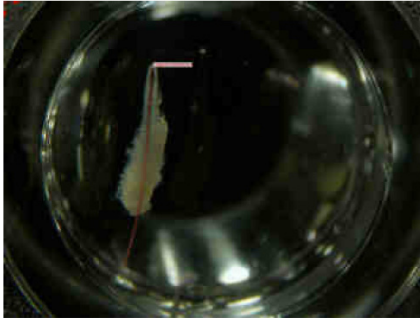
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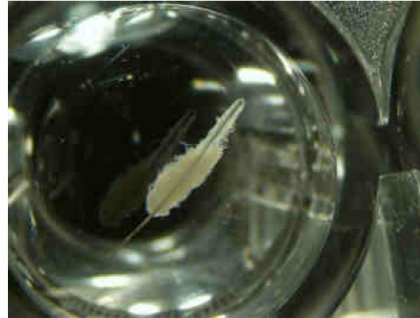
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Record Name: J0\_36



Record Name: J4\_36



Record Name: J8\_36



Record Name: J11\_36

