Hair Growth Factors
A Nanogen Briefing for the Scientific Community
by Toby Cobbledick

Abstract
Emerging research in proteomics and the systems biology approach to molecular biology have significantly increased our understanding of cellular development, and the processes involved in controlling cellular cycling. From this research the importance of the role of cytokines has been established. VEGF in particular has a significant role throughout the development and “life” of a hair follicle, not just to begin growth but to determine differentiation, structure, and the duration of growth. Useful applications of VEGF for hair loss treatment are considered, as well as a method to overcome safety and viability concerns of cytokine therapy.

Introduction
Throughout the history of medicine, we have focussed upon discovering external chemical molecules or elements, and applying them to the body to make a physiological change. It is only very recently that advances in molecular biology, and particularly proteomics, have fully described the use of intercellular signals. We now know that many cellular functions from mitosis and differentiation, right through to apoptosis, are largely controlled by a group of signalling molecules - cytokines.

What is surprising about cytokines is not their existence which has been known to medicine for a long time, but the intensity and breadth of their modes of action. 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 isolated and applied correctly as a therapeutic agent.

Cytokine treatment will be specific with the allergic reactions and unforeseen side effects very unlikely. Any unintended effect would clearly be described by the signal’s action and could be anticipated. Also as cytokines are large and locally acting molecules, they can target small areas effectively.

VEGF and the Hair Growth Cycle
In order to form a hair organ from the dormant dermal papilla cells, three key processes must occur.

Cell Proliferation
Cells of the papilla and secondary hair germ must multiply rapidly. First, the epithelial finger forms into the dermis, the multiplication then reverses direction outwards towards the epidermis. Once formed, stem cells from an as-yet undetermined source proliferate and differentiate into hair matrix cells-identifiable by their high levels of LEF-1 expression[1,2].

The role of VEGF in Anagen is not known, experimentation has shown that dermal papilla cells express high levels of VEGF mRNA in Anagen[3]. This may begin the angiogenic processes required by the hair as an organ later to maintain the cycle, however it is also possible that VEGF may have proliferative effects in certain cells other than endothelial cells.

An additional consideration is the control of LEF-1 expression, proliferation is controlled via a variety of pathways including the MAPKinase pathway. The MAPKinase pathway is stimulated via a calcium dependent signal by VEGF[4]. It is therefore also possible that VEGF indirectly acts as a check on uncontrolled proliferation.

Cell Migration
Proliferation of cells without organisation and migration will not form a hair, or any other organ. A hair shaft is a complex structure, and keratinocyte migration to form the differentiated layered lineages of the hair shaft, as well as the hair bulb and matrix.

A second pathway for VEGF action is commonly known, and upregulates the Akt/PKB genes to prevent apoptosis. However the other function of this pathway upregulates eNOS, producing nitric oxide[5]. This signal causes vasodilation and membrane permeability, and is thought to allow cellular migration and intracellular communication via other cytokines.

In a variety of cells, VEGF also influences cellular migration by stimulating actin reorganisation and focal adhesion turnover[6]. This reorganisation of the cytoskeleton is vital for any cell migration. A similar pathway is stimulated by Wnt11 in other cells, and also utilises the MAPKinase pathway upregulated by VEGF to increase the gene transcription necessary for migration[7].

Angiogenesis
Without doubt the key to maintaining hair shaft growth is a sufficient supply of oxygen and essential amino acids. This requires blood. As has been shown by repeated experiments, increasing blood flow and blood vessel formation to hair follicles improves and maintains hair growth, without this hair growth will cease.

VEGF is the cytokine solely responsible for blood vessel formation. Via the VEGFR2 receptor, VEGF stimulates vascular...
The Na+/K+ATPase channel has another function, regulating Ca²⁺ that maintenance of Ca²⁺ ion levels is necessary for VEGFR2 promote apoptosis through the same pathway[9]. Lowering the concentration of Bcl-2 allows Bax and Bad to interacts with Bax and Bad genes to prevent apoptosis, and so hair loss. It is a hormonal signal which penetrates the follicle, and Dihydrotestosterone has long been studied as a major cause of levels of Ca²⁺ ions to stabilise. As the proliferative and eNOS turnover rate superoxide dismutase removes may reactive antioxidant is superoxide dismutase, as an enzyme with a high signals are degraded rapidly by free radicals, therefore many treatment utilise antioxidants to prolong the signal life. A notable donation of nitric oxide. This gaseous signalling molecule is well Minoxidil is the medicine of choice for most physicians when treating hair loss. It is surprising then that several modes of action have been theorised, but none of them proven. The principle mode of action for minoxidil is thought to be the treatment for alopecia areata. Cytokines and particularly androgenetic alopecia, and diphencyprone is a useful dihydrotestosterone treatments are effective against androgenetic alopecia, and diphencyprone is a useful treatment for alopecia areata. Cytokines and particularly VEGF provide an alternative to these treatments, or supporting role to all of these known therapeutic options.

Apoptosis

Dihydrotestosterone has long been studied as a major cause of hair loss. It is a hormonal signal which penetrates the follicle, and causes downregulation of Bcl-2 leading to apoptosis. Bcl-2 interacts with Bax and Bad genes to prevent apoptosis, and so lowering the concentration of Bcl-2 allows Bax and Bad to promote apoptosis through the same pathway[8]. VEGF blocks Bad conversion, maintaining the pre-apoptotic state[10]. This means that Bcl-2 only has Bax to interact with, and so prevents apoptosis at lower concentrations. VEGF also leads to the downregulation of caspase 9[9], reducing a different apoptotic pathway. VEGF has a clear role in preventing early apoptosis via these two pathways, and the prevention of hypoxia and oxidative stress. This will lead to the maintenance of Anagen for longer.

Treatment Correlations

Minoxidil

Minoxidil is the medicine of choice for most physicians when treating hair loss. It is surprising then that several modes of action have been theorised, but none of them proven.

The principle mode of action for minoxidil is thought to be the donation of nitric oxide. This gaseous signalling molecule is well known to cause vasodilation and improve circulation. Nitric oxide signals are degraded rapidly by free radicals, therefore many treatment utilise antioxidants to prolong the signal life. A notable antioxidant is superoxide dismutase, as an enzyme with a high turnover rate superoxide dismutase removes may reactive oxygen species, and effectively reduces nitric oxide breakdown.

A very credible newer theory is that minoxidil’s effects opening the Na⁺/K⁺ATPase channel promote hair growth; this has been shown by the classification of two channel subtypes in the follicle, one of which is opened by minoxidil. When opened by a different specific channel opener hair growth was improved, when a channel inhibitor was used the growth effect was prevented[11]. The Na⁺/K⁺ATPase channel has another function, regulating Ca²⁺ ion levels. Permanently opening the channels would cause the levels of Ca²⁺ ions to stabilise. As the proliferative and eNOS stimulating effects of VEGF are Ca²⁺ mediated, there is evidence that maintenance of Ca²⁺ ion levels is necessary for VEGFR2 signal transmission to be effective. Therefore minoxidil may make the VEGF signal more efficient and increase the intracellular effect.

An interesting corollary to the possibility of minoxidil acting with or via VEGF is the fact that minoxidil upregulates VEGF expression in Anagen dermal papilla cells. This upregulation ensures adequate vascularisation of the follicle through the Anagen phase, and is likely at least part of the mode of action of minoxidil[12].

Prostaglandins

Prostaglandins have been another widely researched treatment option. Research suggests that prostaglandins are active in the very early stages of Anagen, possibly even at the initiation step as suggested by the new eyelash growth in several clinical trials[19]. The prostaglandin system is complex, made from a large number of different prostaglandins, and still not fully researched.

VEGF has been shown to induce prostaglandin I(2) production in epithelial cells. Prostaglandin I(2) is unlikely to stimulate new hair growth, however prostaglandin I(2) receptors have been found to be specifically expressed in hair cuticle layer, suggesting an important role for hair matrix cell differentiation to form the outer hair cuticle[14]. This outer layer is essential for terminal hair formation.

This may also help explain the necessity for VEGF upregulation in the early anagen stage discussed earlier.

Diphencyprone

Widely regarded as the most successful treatment for alopecia areata, diphencyprone is another treatment with no definite mechanism. As a potent allergen, topical application of diphencyprone as an immunotherapeutic agent stimulates a response and leads to normalisation of hair growth[15].

Recent work shows this “response” is threefold. Firstly, the ratio of CD4/CD8 cells is known to differ significantly in alopecia areata patients. Diphencyprone stimulates a normalisation of this ratio to one approaching normal scalp tissue. Diphencyprone also upregulates the expression of survivin. This helps to preventing the premature apoptosis symptomatic of alopecia areata patients. Lastly, it upregulates the expression of VEGF in hair follicle keratinocytes, maintaining nutrient and oxygen supply[16]. VEGF also has an anti-apoptotic role, downregulating Casp9 and Bad genes which are key to follicle apoptosis[19].

Whilst alopecia areata pathogenesis is still unknown, VEGF explains part of the success of the most successful treatment to date.

In summary, it is well known that minoxidil and anti-dihydrotestosterone treatments are effective against androgenetic alopecia, and diphencyprone is a useful treatment for alopecia areata. Cytokines and particularly VEGF provide an alternative to these treatments, or supporting role to all of these known therapeutic options.
Applications

There are clearly diverse possibilities for VEGF, either as an independent treatment or to supplement minoxidil for androgenetic alopecia, or diphenycprone for alopecia areata. As the role in stimulating vascularisation is clear, it is also likely that a solution containing VEGF would help graft survival and improve wound healing times after surgery.

It is also conceivable that VEGF could be added alone, or with other growth factors to an autologous growth factor treatment such as Platelet Rich Plasma therapy.

VEGF is quite a large molecule, and applied topically to normal human epidermis, penetration would be low. Research into molecule penetration has shown that penetration via the hair shaft is possible. Penetration through the thinner epidermis around pores and hair shafts would be sufficient to allow a useful amount of VEGF to work.

As has been widely discussed, a way to ensure penetration of any larger molecule through the scalp is to use a microneedle roller. The microneedle array creates quickly healing channels through the stratum corneum, allowing over 5 times penetration of many molecules, including proteins far larger than VEGF\(^\text{17}\). This will deliver effective levels of VEGF from solutions containing 1ppm sh-VEGF.

Safe, Legal Growth Factors

There are many safety and ethical concerns with cytokines, however these can be overcome with careful selection of sources and rigorous purification processes.

These concerns have been widely explored by the media, ensuring that it is not just the physician’s position that is important. Patients will have their own opinions on the subject, and will judge their physician in light of these opinions. Unfortunately this is true even if the patient’s opinion has largely been formed by the media, and consequently the patient may not have fully understood the evidence and rationale behind the physician’s position. With this in mind the following considerations are very important.

Embryology

The most obvious source of human growth factors is human cells. These can be grown successfully from human embryos, stem cells can be used, or guided to differentiate into suitable cell lines. These can then be kept in conditions to produce growth factors which are then extracted and purified.

Whist there are advantages to this method, it is costly. Also many people object to the use of embryos for research, and in fact an international moratorium has been declared limited research with human cells. Apart from ethical considerations, it is also illegal in many countries to use any human tissue or human-derived product for medical treatment, with the exception of organ transplants.

A further consideration behind using human-derived growth factors is the danger presented by prion proteins. The dangers of these causing Creutzfeldt - Jakob disease have been widely publicised. The dangerous presence of prion proteins is possible in any tissue, and is the main reason behind the widespread ban on human-derived products.

Bacterial Sources

One solution to the problem would be to use recombinant bacteria such as E. coli to produce the desired growth factor. There are however, several drawbacks to bacterial systems.

Recombinant technology would utilise human DNA and incorporate that, via a plasmid or viral vector, into a bacterial cell. The bacterial cell therefore contains human DNA, and even when purified the end product may still contain traces of this, making it a human-derived product with many of the associated dangers and legal obstacles.

Bacteria are prokaryotes and as such lack many of the cell components of eukaryotic cells, notably those responsible for folding complex proteins. Therefore even a genetically perfect protein is often not assembled correctly, giving lower or no biological activity.

Cytotoxins are commonly produced waste products of most prokaryotes. They are mostly small soluble glycoproteins and other molecules that do not harm the organism in small quantities; however they can have noticeable effects on humans. Due to their nature, cytotoxins are not removed by purification processes, and therefore remain in the finished product. These can cause side-effects or prevent the cytokine from working correctly in vivo.

Yeast

Fungi are the next most obvious source to make recombinant growth factors. They are eukaryotes and very similar to human cells, and so produce active proteins very similar to the human version.

There still remains the problem of small traces of human material, however, which mean many yeast products are only legal for research purposes.

Although less of a concern, yeast cells produce cytotoxins like bacterial systems. Like bacterial cytotoxins, these can cause side-effects and allergic-type reactions.

Even the traces of fungal tissue in purified growth factor media have the potential to stimulate immune responses. This is especially important as human epithelial tissues react strongly to many varieties of fungi as an evolved response to fungal infection. The stimulation of background immune responses can change or cancel out the effects of growth factor treatment.


**sh-VEGF**

Synthetic Human VEGF or sh-VEGF is not actually chemically synthesised, it is produced for Nanogen utilising a unique plant expression system.

First, the human gene is immobilised and sequenced, the sequence is the de-novo synthesised, and replicated by PCR. This creates only newly synthesised genes without even a molecule derived from a human.

The PCR copied genes are purified and transfected into *Hordeum vulgare* plant seeds. The plants are then grown hydroponically to ensure conditions are perfect for VEGF production, and prevent the modified genes from spreading to the environment. The high-yield *H. vulgare* is then harvested and the VEGF purified.

As a plant is also a eukaryote, the identical VEGF protein is produced and folded correctly, and so has homology with the original human protein, and high activity.

*H. vulgare* does not produce cytotoxins like bacteria and yeast, and does not stimulate an immune response. This prevents side-effects and reactions, ensuring patient safety and results.

**Nanogen sh-VEGF Complex™**

Safe, plant derived sh-VEGF has been exclusively developed with Nanogen as a research partner. All Nanogen treatment products contain the patent pending Nanogen sh-VEGF Complex™, a complex of sh-VEGF and ciclopirox olamine. Ciclopirox olamine supports the action of VEGF, upregulating VEGF expression in hypoxic cells where VEGF is needed most[19]. Additionally, ciclopirox olamine may maintain the Ca²⁺ ion levels in the cell, ensuring signal transmission from the VEGFR2 receptor throughout the cell.

**References**

Intellectual Property

Technology and Intellectual Property Outline

**Nanogen® sh-VEGF Complex™**
A UK research patent application with international priority. The application protects sh-VEGF complex and its various applications for promoting hair growth. The application discusses use of the complex alone, in combination with known medicines and autologous growth factor therapy, and in combination with skin needling devices. Possible uses of the complex are in treatment shampoos, conditioners, and serums. Clinical applications include pre and post operative serums for patients, and graft treatment solutions.

**Nanofibres® Jar**
Application filed internationally. The Nanofibres Jar has several patented features. The electrostatic strip, materials and internal design of the jar are discussed by the application, with their various effects of controlling and increasing electrostatic charge of the fibres. The Nanofibres Jar is also subject to an EU Protected Design and a US Design Patent.

**Nanofibres® Coating**
A UK patent application with international priority. The application primarily protects a coating applied to the surface of the fibre. The coating allows the fibre to become dipolar charged. The application also covers optimal shape and size configurations for dipolar charging. The application further discusses the utility of a dipolar charged fibre, the perpendicular binding and the increased likelihood of binding in various electrostatic conditions.

**Electrostatically Compatible Shampoos and Conditioners (Hair Prepare™, Daily Volume™, Follicle Defence™)**
A UK patent application with international priority. The application protects a combination of amphoteric surfactants and dipolar amino acids which mimic the hair’s natural surface charge. The application discusses benefits of maintaining or mimicking the hair surface charge to improve hair concealing fibre binding.

**Hydroguard™ for Locking Mist Plus®**
A UK patent application with international priority. The application discusses a combination of water resistant polymers. The polymer combination would act to adhere a hair concealing fibre to the hair. Additionally the polymer combination would insulate the fibre to protect the bond to the hair surface. The application discloses a number of embodiments for the combination of polymers, including a fixative spray for hair concealing fibres and scalp colourants.

**Scalproller™**
Application filed internationally. The application discusses several features to maintain sterility and safety of the microneedle array. The application additionally protects several means to reduce or control pain and inflammation during use. Whilst applied for, this patent is unpublished and further details are not disclosed at this time.

**Aquamatch™**
UK patent applications with international priority. The formula marketed as “Aquamatch” is subject to several patents pending. Details are not disclosed as the applications are not published at this time.

**Scalp Ease MDL™**
UK patent applications with international priority. The formula marketed as “Scalp Ease MDL” is subject to several patents pending. Details are not disclosed as the applications are not published at this time.

**Intervention™**
UK patent application with international priority. Two specific formulae are protected by this application. The formulae discussed contain various combinations of ingredients that aim to reduce hair loss and maintain hair growth. Several embodiments are considered, including an oral tablet format.

**Trademarks**
Nanogen, Scalp Ease MDL, Scalproller, Aquamatch, Nanofibres and Locking Mist Plus are registered trademarks or in the process of trademark registration. All other product, complex and other names marked with the ™ symbol are used as trademarks, and may be currently under trademark registration.

**Copyright**
All images, diagrams, and text written for Nanogen are copyright material, and cannot be reproduced without consent.