QR 678 hair growth factors formulation - In vivo cellular toxicity & In vivo animal efficacy study.

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Running title: Efficacy & safety of QR 678 hair growth factors formulation

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Abstract

Background: We have demonstrated the efficacy of a recombinant, bioengineered, hair growth factor formulation (QR 678), in causing hair regrowth in humans. We now demonstrate safety analysis of QR 678 formulation, using an in vitro cytotoxicity assay. We also evaluate the efficacy of hair growth, using intradermal injections, into animal skin.

Objective: The objective of this study was to assess the in vitro safety & in vivo efficacy of the QR 678 formulation.

Material & method:

The formulation contains Vascular Endothelial Growth Factor (VEGF), Basic

Fibroblast Growth Factor (bFGF), Insulin like Growth Factor-1 (IGF-1), Keratinocyte Growth Factor (KGF), Thymosin β4 & Copper tripeptide 1, suspended in a sterile injectable vehicle.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was employed to explore the cytotoxic effects of each factor used in the composition, in Human keratinocyte Cell & human fibroblast cell assay.

In vivo analysis, wherein study animals were given intradermal QR 678 injections, were conducted, to assess if the formulation produces hair growth. Intradermal injections with cytosine arabinoside, were also used to evaluate the growth.

Results: A positive response was observed with respect to the number of mice exhibiting hair growth at the injection sites. The injections caused retention of hair in a 0.25 cm radius around the injection site, in animals on treatment with the chemotherapeutic agent.

Conclusion: Intra-dermal injections of QR 678 hair growth factor formulation is safe & efficacious option for alopecia. Results seem encouraging enough to warrant a trial in human patients with secondary alopecia, post cancer chemotherapy.

Introduction:

The most common cause of baldness or hair loss (95%) is Androgenetic alopecia.[1] The current surgical, medical and cosmetic interventions are limited in approach &

success.[2] There are several growth factors which have been found to stimulate or inhibit different stages in the hair growth cycle. Various growth factors studied for hair follicle growth are vascular endothelial growth factor (VEGF) [2], epidermal growth factor (EGF), Insulin 1-like growth factor (IGF), Fibroblast growth factor (FGF) [3], Wingless-related integration site (Wnt), noggin, Keratinocyte Growth Factor, Copper Tripeptides, and more. These growth factors can be safe, cheap, & non-allergenic tools, in the management of alopecia. [4, 5, 6]

We have prepared a bioengineered, recombinant formulation, consisting of a combination of growth factors, called the QR 678 hair growth factor formulation. A QR Code is a code used in medicine derived from “Quick Response”. 678 in Morse Code signifies “there is no answer”. This formulation has been named QR 678 to signify a “Quick Response to a disease which earlier had no answer” i.e. to Alopecia. This is administered intradermally into the scalp skin and is capable of preventing hair loss and stimulating hair growth. The first in human trial to prove its safety and efficacy demonstrated significant reduction in hair fall in 83% patients, on hair pull test. Videomicroscopic image evaluation showed that most patients had a decrease in the number of vellus hairs, increase in number of terminal hairs and increase in shaft diameter. 75% patients believed that the hair injections were aiding the treatment of their hair loss, and it was also beneficial in post-hair transplant patients. At one year, a statistically significant increase in total hair count (P=0.002) continued to be seen. The treatment was well tolerated. [7,8]

Before conducting this pilot human study, the formulation was tested on cellular assays and animal models to establish the optimum concentration of the multiple

growth factors, it's safety & the efficacy. And the current article is based on the same animal study mentioned above.

Reported therapeutically acceptable ranges of the growth factors used are as below:

i. Vascular endothelial growth factor (Human oligopeptide-11) – 0.01mg/L – 100mg/L

ii. Basic fibroblast growth factor (Human oligopeptide-3) – 0.01mg/L – 100 mg/L

iii. Insulin like growth factor (Human oligopeptide-2) - 0.01mg/L – 100mg/L

iv. Copper tripeptide 1 – 0.1mg/L – 500 mg/L

v. Keratinocyte growth factor – 0.01mg/L – 100 mg/L

vi. Thymosin β4 – 0.005mg/L – 100 mg/L

Materials and Methods

I) Determination of the cytotoxic effects of the individual growth factors to determine safe levels.

Method : The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [9] was employed to explore the cytotoxic effects of each of the growth factors used in the composition, in Human keratinocyte Cell and Human fibroblast cell.

II) Preparation & physicochemical characterization of the growth factor formulation

In a preferred embodiment, the growth factor formulations included the growth factors

in the concentrations, as given in in **Table 2**, additionally along with vitamins, minerals, nucleic acids and amino acids, diluents and/or carriers along with pharmaceutically acceptable diluents &/or carriers.

The formulations as described were formulated for intradermal injection to the treatment area. Suitable vehicles for injection include, but are not limited to saline and distilled water.

Preparation of the formulation:

Adequate concentrations of VEGF, Basic FGF, IGF-1, Copper tripeptide 1, KGF and Thymosin β4 additionally with pharmaceutically/cosmetically acceptable and appropriate dose of vitamins, minerals, amino acids and nucleic acids were added to 1 litre of distilled water, to yield the concentrations of the solutions as shown in table 2. The formulation was then biologically sterilized and bottled into vials of 5 millilitre each. The formulation was stable at all concentrations of growth factors and could be stored at room temperature (below 25°C).

In vivo Study Arm 1: Stimulation of hair growth by representative growth factors & biomimetic peptides in mammals

Method: The following study arm evaluated stimulation of hair growth in warm blooded animals, after intradermal injection of representative growth factors and peptides of the formulation.

In this experiment, C3H mice were divided into 5 groups. The backs of the C3H mice, (60 days old, telogen hair growth phase) were closely clipped on day 1, with an electric clipper. A sterile solution of the chosen formulation concentration was then

injected intradermally (i.e.: infiltrated within the skin) at 2 locations within the clipped areas of the mice. Injection at 2 locations provided 2 test locations within the clipped area of each mouse. Each injection (0.1 ml) contained different proportions of IGF 1, VEGF, bFGF, KGF, Thymosin β4 and copper tripeptide-1, in distilled water, labeled as solutions 1 through 4. A group of saline injected mice (0.1 ml) served as controls. (**Table 2**)

In vivo Study Arm 2: hair follicle viability by intradermal injection of the pharmaceutical composition in secondary alopecia

Method: The following study arm evaluated maintenance of hair follicle viability by intradermal injection of the pharmaceutical composition in secondary alopecia.

The following experiment illustrated the localized maintenance of hair follicle viability (growth) by intradermal (local) injection of the pharmaceutical composition during treatment with chemotherapeutic agent cytosine arabinoside (Ara-C).

In this experiment, Sprague Dawley rat pups aged 8 days were maintained in 5 litters (n=10-12 per litter) for the duration of the study. On day 0, the litters received intradermal injection of the pharmaceutical composition in distilled water (solutions 1/2/3 or 4 as described in the example 3), or a saline control (1 injection per animal, 0.05 ml per injection). Each litter contained 2 normal control animals which received neither the pharmaceutical composition nor Ara-C, they received saline injection only. On day 1, the designated animals began a series of 7 consecutive daily intrapertioneal injections of Ara-C 25mg/kg. On day 10, all animals were evaluated for the extent of hair loss at the injection sites. Using the rating identified as below:

<u>Grade Degree of alopecia</u>

0 normal (no loss of hair)

1 slight thinning

2 moderate thinning

3 sparse hair cover

4 total loss of hair.

Results:

I) Determination of the cytotoxic effects of the individual growth factors to determine safe levels.

i) In vitro, human keratinocyte cell (**graph 1**) as well as human fibroblast cell (**graph 2**) : bFGF is SAFE (No Cellular Toxicity) up to tested 10ppm (μg/ml) - Reported oral toxicity, Rat : LD50 > 10,000 mg/kg.

ii) In vitro, human keratinocyte cell (**graph 3**) & human fibroblast cell (**graph 4**) IGF-1 is SAFE (No Cellular Toxicity) up to tested 10ppm (μg/ml) - Reported oral toxicity, Rat : LD50 > 10,000 mg/kg.

iii) In vitro, human keratinocyte cell (**graph 5**) & human fibroblast cell (**graph 6**) : KGF is SAFE (No Cellular Toxicity) up to tested 10ppm (μg/ml) - Reported oral toxicity, Rat : LD50 > 10,000 mg/kg.

iv) In vitro, human keratinocyte cell (**graph 7**) & human fibroblast cell (**graph 8**) : VEGF is SAFE (No Cellular Toxicity) up to tested 10ppm (μg/ml) - Reported oral toxicity, Rat : LD50 > 10,000 mg/kg.

v) In vitro, human keratinocyte cell (**graph 9**) & human fibroblast cell: (**graph 10**)

Thymosin-B4 is SAFE (No Cellular Toxicity) up to tested 10ppm (μg/ml) - Reported oral toxicity, Rat : LD50 > 10,000 mg/kg.

vi) In vitro, human keratinocyte cell (**graph 11**) & human fibroblast cell (**graph 12**): Copper Tripeptide 1 is SAFE (No Cellular Toxicity) up to tested 10,000ppm (μg/ml) - Reported oral toxicity, Rat : LD50 > 10,000 mg/kg

vii) Toxicity data of the growth factors used in the composition is seen in **Table 1**

In vivo Study Arm 1: Stimulation of hair growth by representative growth factors & biomimetic peptides in mammals

With injection of the above intradermal pharmaceutical formulation, indications of hair growth were seen within 10 days. The first visual signs were darkening of the skin in a circular region surrounding the injection site. The size of this region was generally dose dependent, increasing with an increase in dose of the growth factors, to a certain extent. The 0.1 ml injections used in this experiment produced a circle of hair growth measuring approximately 0.5 cm^2 to 5 cm^2 in diameter. Active hair growth occurred between 14-20 days of the injection, with a maximum density seen on day 30. Both the number of mice growing hair at the injection site & the diameter of hair growth region were determined on day 21. A positive response was observed with respect to the number of mice exhibiting hair growth at the injection sites, compared to the total number of mice injected in the study. The results of this experiment are presented in **Table 3** (The day of onset is the day at which hair follicle pigmentation was first observed).

Solution 3 comprising 0.0002 mg/0.1 ml of IGF1, 0.0002 mg of bFGF, 0.0005 mg of

VEGF and 0.0001 mg of KGF, 0.001 mg of copper tripeptide and $1x10^{-6}$ mg of Thymosin β4 within distilled water gave the best response. Increase in concentration of the ingredients beyond that in solution 4 did not give any significant benefit in terms of number of mice growing hair or the diameter of hair growth region. None of the mice in the group injected with solution 5, died or showed any other signs of clinical or cellular toxicity. This proved the safety of the composition, even with very high doses of the growth factors.

In vivo Study Arm 2: hair follicle viability by intradermal injection of the pharmaceutical composition in secondary alopecia

Ara-C injections caused significant hair loss by day 5-6 in most animals. In order to evaluate the stimulatory effects of the intradermal QR678 formulation, the degree of hair loss was evaluated at the injected site daily. Injections generally caused retention of hair in a 0.25 cm radius around the injection site, most notably in the solution 3 group.

Table 4 presents the results as evaluated on day 10 using the previously described rating scale, with the degree of alopecia being expressed as the average response seen at the site of injection.

The observation of retained hair within the area of injection was examined histologically. While normal appearing & functional anagen hair follicles were observed at the site of injection of the hair formulation, follicles located away from the injection were dystrophic & nonfunctional (disruption of the integrity of inner and

outer root sheaths, & disrupted hair shafts). This data confirmed the gross observation of normal hair follicular function within the site of QR678 injections, & illustrated the stimulatory effect of the intradermal injections on hair follicles, which maintains the active hair growth cycle during chemotherapy treatment.

Discussion

Hair growth factors, when used in combination, have been shown to have a synergistic impact on human hair growth.[7] Herein, we publish the results of the pre-clinical data on file, demonstrating the safety & efficacy of the hair formulation, which we call the QR 678 hair growth factor injections.

To evaluate safety, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [9] was employed to explore the cytotoxic effects of each of the growth factors used in the composition, in Human keratinocyte Cell and human fibroblast cell. All the tested individual growth factors were extremely safe, with no cellular toxicity being demonstrated at high concentrations. IGF 1, VEGF, bFGF, KGF & Thymosin β4 is safe, with no cellular toxicity up to tested 10ppm. Copper tripepetide was safe, with no cellular toxicity up to tested 10000 ppm.

To evaluate efficacy, we evaluated the stimulation of hair growth in C3H, after intradermal injection of various concentrations of the growth factors & peptides of the formulation. Solution 3 comprising 0.0002 mg/0.1 ml of IGF1, 0.0002 mg of bFGF, 0.0005 mg of VEGF, 0.0001 mg of KGF, 0.001 mg of copper tripeptide and $1x10^{-6}$ mg of Thymosin β4, within distilled water, gave the best response. Increase in concentration of the ingredients beyond that in solution 4, did not give any significant

benefit in terms of number of mice growing hair or the diameter of hair growth region. None of the mice in the group injected with solution 5, died or showed any other signs of clinical or cellular toxicity. This proved the safety of the composition, even with very high doses of the growth factors. This formulation used in solution 3 was called the QR 678 hair growth factor formulation (table 3) & the was used in the human trial subsequently.[7]

Efficacy was further evaluated in another study arm, which evaluated maintenance of hair follicle viability by intradermal injection of the QR 678 formulation, in secondary alopecia. This experiment illustrated the localized maintenance of hair follicle viability (growth) by intradermal QR 678 formulation injections, during treatment with chemotherapeutic agent cytosine arabinoside (Ara-C). Injections generally caused retention of hair in a 0.25 cm radius around the injection site, most notably in the solution 3 group. The observation of retained hair within the area of injection was examined histologically. While normal appearing & functional anagen hair follicles were observed at the site of injection of the QR 678 hair formulation, follicles located away from the injection were dystrophic & nonfunctional (disruption of the integrity of inner and outer root sheaths, & disrupted hair shafts). This data confirmed the gross observation of normal hair follicular function within the site of QR678 injections, & illustrated the stimulatory effect of the intradermal injections on hair follicle, which resulted in maintainance of the active hair growth cycle during chemotherapy treatment.

VEGF, essential for angiogenesis & vascular permeability, may be responsible for maintaining proper vasculature around the hair follicle, during the anagen growth

phase. [10,11] KGF is highly capable of counteracting chemotherapy induced alopecia & it is one of the components of our formulation. [12] IGF-I is critically involved in promoting hair growth by regulating cellular proliferation & migration during the development of hair follicles. IGF-I has been reported to prevent the follicle from developing catagen- like status. [13,14, 15] Thymosin B4 promotes hair growth in various rat & mice models, including a transgenic thymosin B 4 overexpressing mouse, by influencing follicle stem cell growth, migration, differentiation, & protease production. [16] The bFGF has been found to promote hair growth by inducing the anagen phase in resting hair follicles & has been considered to be a potential hair-growth promoting agent. [17] The effects of L alanyl L histidyl L lysine Cu2+ (AHK-Cu) copper tripeptide on human hair growth ex vivo & cultured dermal papilla cells was investigated & shown to promote the growth of human hair follicles. [18]

In summary, we publish the results of the pre-clinical data on file, demonstrating the safety & efficacy of intrdermal QR 678 hair growth factor injections. The findings of cellular assays & animal studies suggest that this QR 678 formulation is safe & efficacious in treating hair loss in mammals. The results seem encouraging enough to warrant a trial in human patients with secondary alopecia, post cancer chemotherapy.

References:

1) Rogers NE, Avram MR. Medical treatments for male & female pattern hair loss. J

Am Acad Dermatol. 2008; 59: 547-66.

2) Zimber MP, Ziering C, Zeigler F, Hubka M, at al. Hair regrowth following a Wnt- & follistatin containing treatment: safety & efficacy in a first-in-man phase 1 clinical trial. J Drugs Dermatol. 2011; 10:1308-12.

3) Philpott MP, Sanders DA, Kealey T. Effects of insulin & insulin-like growth factors on cultured human hair follicles: IGF-I at physiologic concentrations is an important regulator of hair follicle growth in vitro. J Invest Dermatol. 1994; 102: 857-61.

4) Price VH. Treatment of hair loss. N Engl J Med. 1999; 341: 964–73.

5) Ebling FG, Hale PA, Randall VA. Hormones & hair growth. In: Goldsmith LA (ed) Biochemistry & physiology of the Skin, 2nd ed, Clarendon, Oxford. 1991: 660-90.

6) Parsley WM, Perez-Meza D. Review of factors affecting the growth & survival of follicular grafts. J Cutan Aesthet Surg. 2010; 3:69-75.

7) Kapoor R, Shome D.Intradermal injections of a hair growth factors formulation, for enhancement of human hair regrowth - Safety & Efficacy Evaluation in a First-in-Man Pilot Clinical Study. J Cosm and Las Ther. 2018 Feb 26:1-11. doi: 10.1080/14764172.2018.1439965. [Epub ahead of print]

8) Kapoor R, Shome D. Stem cells, PRP or growth factors like the QR678 - what really works in non surgical hair growth? International Master Course on Aging Skin meeting. Mumbai, 2016.

9) https://en.wikipedia.org/wiki/MTT_assay

10) Kiichiro Yano, Lawrence F. Brown, & Michael Detmar; Control of hair growth & follicle size by VEGF-mediated angiogenesis J. Clin. Invest 2001; 107:409-17.

11) Kozlowska U, Blume-Peytavi U, Kodelja V et al. Expression of vascular

endothelial growth factor (VEGF) in various compartments of the human hair follicle. Arch Dermatol Res. 1998; 290:661-8.

12) Danilenko DM, Ring BD, Yanagihara D et al. Keratinocyte growth factor is an important endogenous mediator of hair follicle growth, development, & differentiation. Normalization of the nu/nu follicular differentiation defect & amelioration of chemotherapy-induced alopecia. Am J Pathol. 1995; 147: 145-54.

13) Barber BL, Kaufman KD, Kozloff RC, Girman CJ, Guess HA. A hair growth questionnaire for use in the evaluation of therapeutic effects in men. J Dermatol Treat. 1998; 9:181–6.

14) Jones JI, Clemmons DR. Insulin-like growth factors & their binding proteins: biological actions. Endocr Rev. 1995; 16:3-34.

15) Lindner G, Botchkarev VA, Botchkareva NV et al. Analysis of apoptosis during hair follicle regression (catagen). Am J Pathol 1997; 151:1601-17.

16) Philp D, St-Surin S, Cha HJ et al. Thymosin beta 4 induces hair growth via stem cell migration & differentiation. Ann N Y Acad Sci. 2007; 1112: 95-103.

17) Lin WH, Xiang LJ, Shi HX et al. Fibroblast Growth Factors Stimulate Hair Growth through β-Catenin & Shh Expression in C57BL/6 Mice. BioMed Res Int. 2015; 2015: 730139.

18) Pyo HK, Yoo HG, Won CH et al. The Effect of Tripeptide-Copper Complex on Human Hair Growth In Vitro. Arch Pharm Res. 2007; 30: 834-9.

Table Captions:

1) Toxicity data of the growth factors used in the formulations.
2) Solutions with varied concentrations of growth factors injected in mice in in

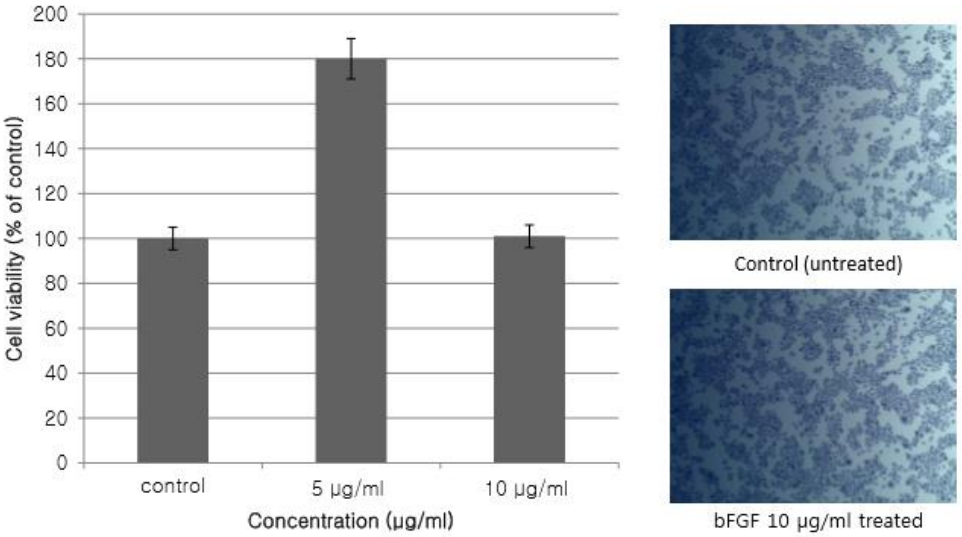
vivo study 1.

3) Hair growth response seen in mice in in vivo study 1.
4) Localized maintenance of hair follicle viability (growth) by intradermal (local) injection of the invented composition during treatment with chemotherapeutic agent cytosine arabinoside (Ara-C), as evaluated on day 10 of injection.

Graph Legends:

1) Cytotoxic effect of bFGF on Human keratinocyte cells in vitro
2) Cytotoxic effect of bFGF on Human fibroblast cells in vitro
3) Cytotoxic effect of IGF-1 on Human keratinocyte cells in vitro
4) Cytotoxic effect of IGF-1 on Human fibroblast cells in vitro
5) Cytotoxic effect of KGF on Human keratinocyte cells in vitro
6) Cytotoxic effect of KGF on Human fibroblast cell in vitro
7) Cytotoxic effect of VeGF on Human keratinocyte cells in vitro
8) Cytotoxic effect of VeGF on Human fibroblast cell in vitro
9) Cytotoxic effect of Thymosin B4 on Human keratinocyte cells in vitro
10) Cytotoxic effect of Thymosin B4 on Human fibroblast cells in vitro
11) Cytotoxic effect of Cu GHK on Human keratinocyte cells in vitro
12) Cytotoxic effect of Cu- GHK on Human fibroblast cells in vitro

Graph 1)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120
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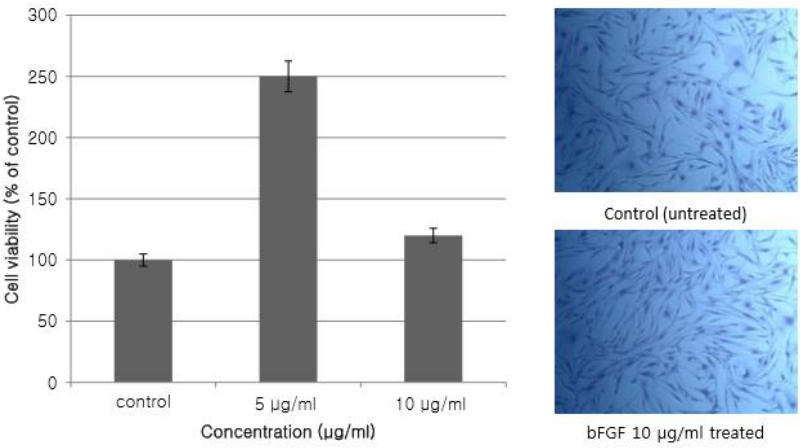
10 µg/ml
Concentration (µg/ml)
Control (untreated)
bFGF 10 µg/ml treated

Graph 2)


300
250
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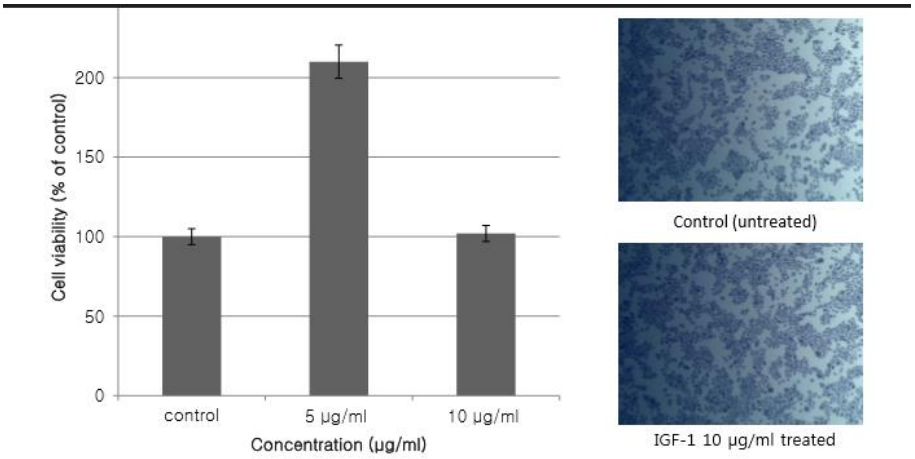
Cell viability (% of control)
control
5 µg/ml
10 µg/ml
Concentration (µg/ml)
Control (untreated)
bFGF 10 µg/ml treated

Graph 3)



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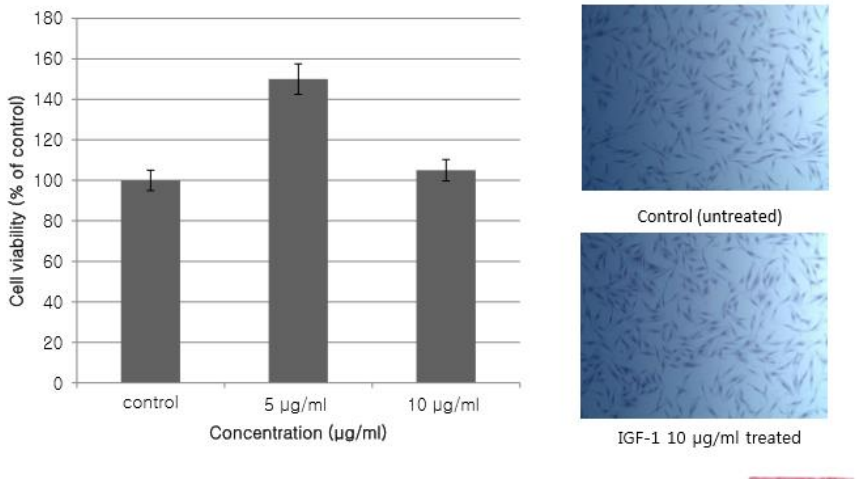

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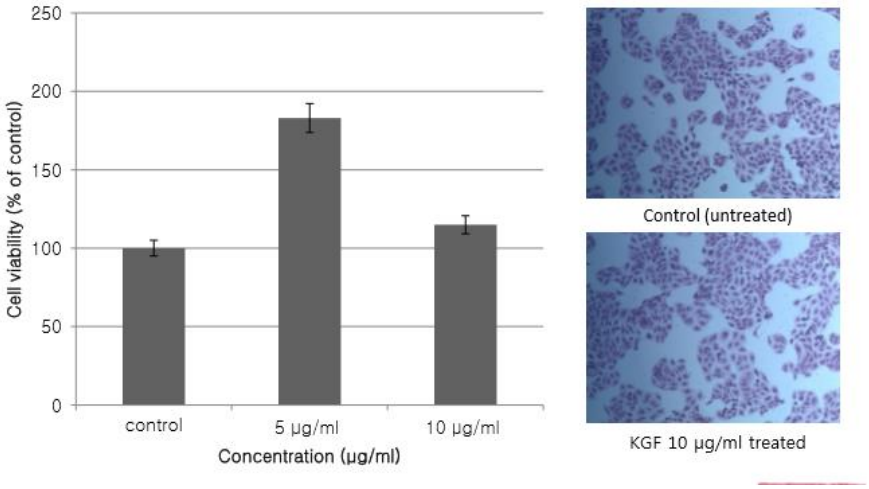

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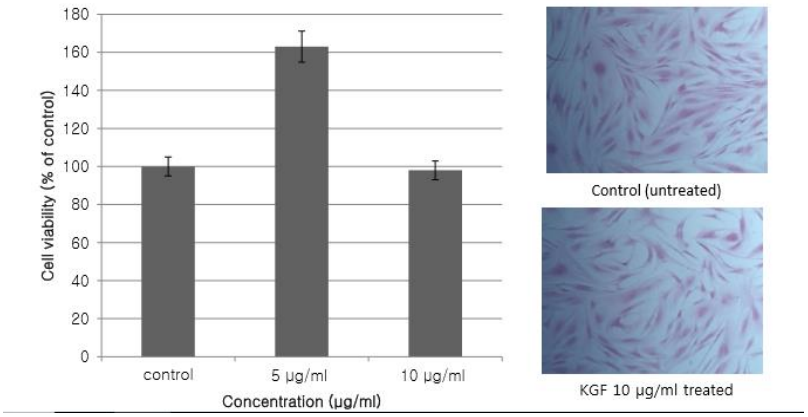

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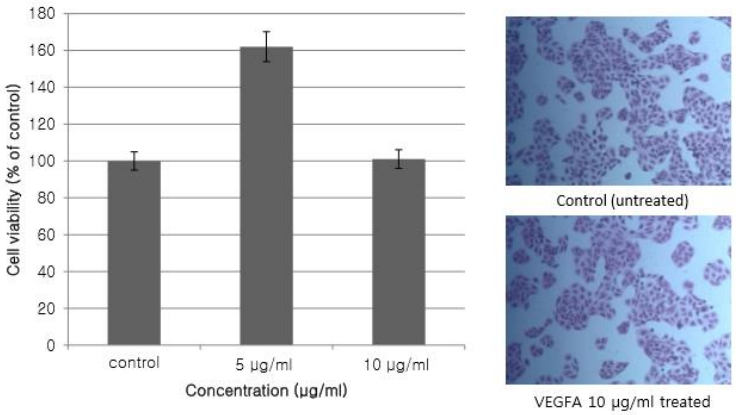

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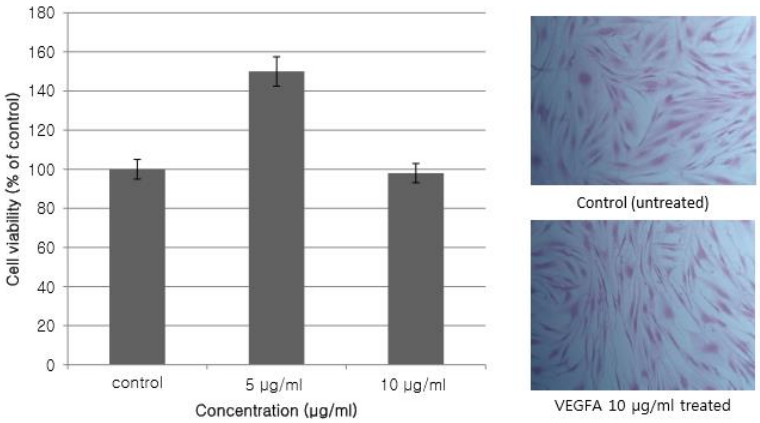

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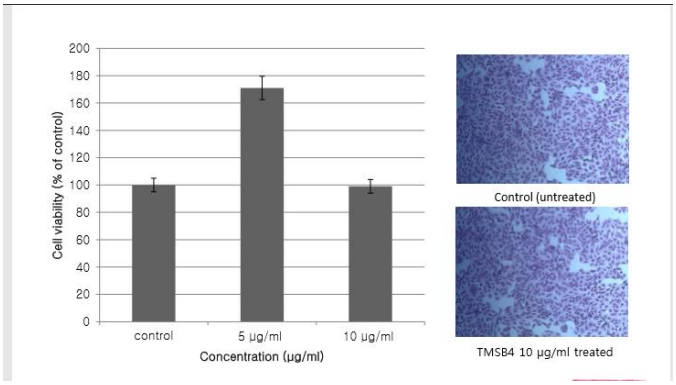

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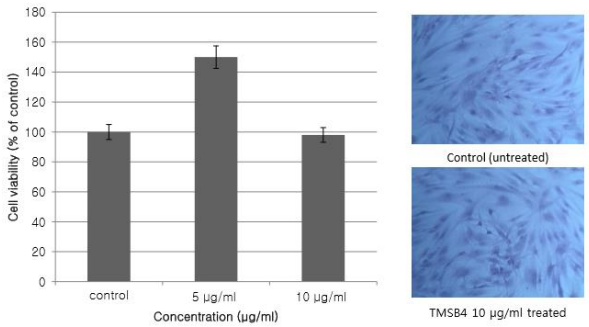

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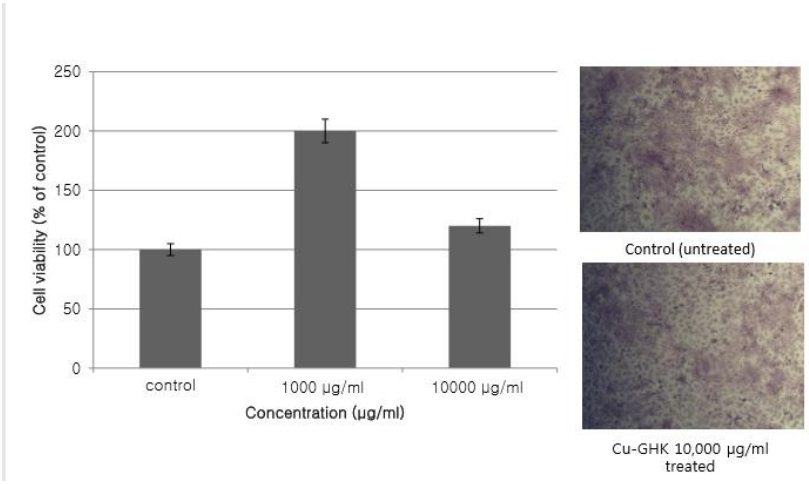

Graph 12)

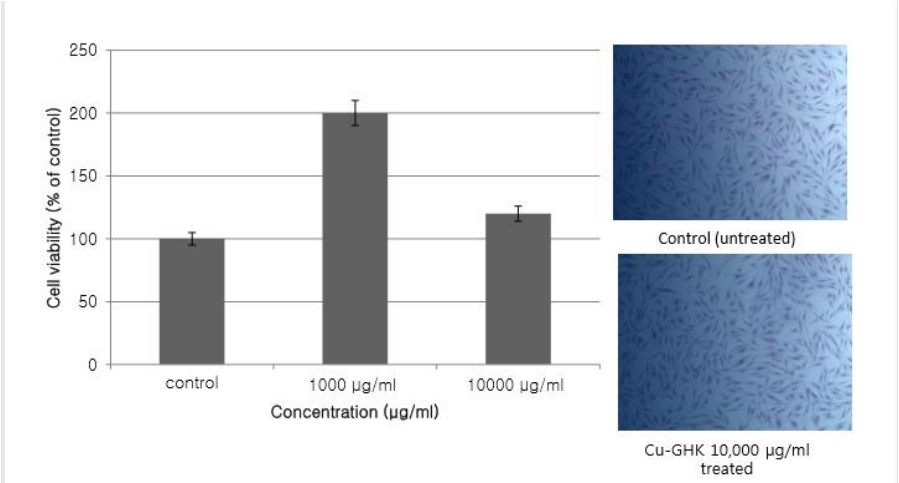

Table 1)

	Toxicity data	Reference
IGF-1	ED_{50}: ≤10.0 ng/ml using mouse Balb/3T3 cells.	Ref. 1 (IGF-1)
	Endotoxin Level: <0.10 EU per 1 μg of the protein by the LAL method.	Ref. 1-2 (IGF-1)
bFGF	Rat, Oral, LD50 : 400mg/kg Mouse, intramuscular, LD50: 108mg/kg. Effect : Behavioural : convulsions or effect on seizure threshold. Mouse intraperitoneal, LD50 : 154mg/kg.	Ref. 2 (bFGF)
	Endotoxin Level: <0.10 EU per 1 μg of the protein by the LAL method.	Ref. 2-1 (bFGF)
KGF	Endotoxin Level : <0.10 EU per 1 μg of the protein by the LAL method.	Ref. 3 (KGF)
VEGFA	Endotoxin Level: <0.01 EU per 1 μg of the protein by the LAL method.	Ref. 4 (VEGFA) Ref. 4-1 (VEGFA)
TMSB4	Endotoxin Level: < 0.1 ng/μg of protein (<1EU/μg).	Ref. 5 (TMSB4) Ref. 5-1 (TMSB4)
Noggin	Endotoxin Level: <0.10 EU per 1 μg of the protein by the LAL method.	Ref. 6 (Noggin)
Copper Tripeptide-1	Acute toxicity : LD50 mouse (I.P.) :=160mg/kg, (I.V.)=110-120mg/kg Rat(I.V)≥75mg/kg, Rat(oral)≥150mg/kg	Ref. 7 (Noggin)

Table 2:1mg/L means 1 ppm

	VEGF (mg/L)	bFGF (mg/L)	IGF -1 (mg/L)	Cu tripeptide 1 (mg/L)	KGF (mg/L)	Thymosin β4 (mg/L)
Solution 1	0.01	0.01	0.01	0.1	0.01	0.005
Solution 2	2	1	1	5	0.5	0.001
Solution 3	5	2	2	10	1	0.01
Solution 4	15	5	5	30	2	0.1
Solution 5	50	20	20	100	10	1

Table 3:

Composition	Number of animals growing hair	Day of onset	Area of hair growth around the injection site
Solution 1	2/5	10	< 1 cm diameter
Solution 2	3/5	10	> 1 cm diameter
Solution 3	5/5	10	> 1 cm diameter
Solution 4	4/5	10	> 1 cm diameter
Solution 5	4/5	10	>1cm diameter
Solution 6(saline control)	0	NA	NA

Table 4:

Composition	n=	Degree of alopecia (mean)
Saline only	8	0.0
Saline + Ara-C	8	4.0
Solution 1 + Ara-C	8	3.25
Solution 2 + Ara-C	8	2.38
Solution 3 + Ara-C	9	1.44
Solution 4 + Ara-C	9	1.11