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

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Abstract:	Background: Androgenetic alopecia is the most common cause of hair loss. The current modalities are limited in approach & success. QR678 ® is a bioengineered, recombinant formulation, consisting of a combination of growth factors. This study demonstrates safety analysis of QR678 ® formulation, using an In vitro cytotoxicity assay. The study also highlights the In vivo efficacy of the QR 678 ® formulation. Material & method: Factor like 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. Also hair follicle viability was checked by intradermal injection of the pharmaceutical composition in secondary alopecia. 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. On cytotoxicity study, all the factors were found to be safe in Human keratinocyte Cell & human fibroblast cell assay. It was demonstrated a positive response 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.

Response to Reviewers:	Have uploaded the high resolution images as per the editor's comment

07-02-2020

Editor-in-Chief,

Plastic and Reconstructive Surgery Global Open

Respected Editor,

We have submitted our paper on a novel mechanism of hair growth, tested on an in-vitro cellular toxicity model and an in-vivo animal model.

This particular hair growth formulation has also been tested in a subsequent human trial which has been published.[7] So in some sense, the current submission is a PREQUEL of a product, which has already passed muster in human trials. This hair growth formulation already has a USA and Indian Patent approvals and is FDA approved in multiple countries for hair growth. The submission needs to be seen in that light.

While we appreciate that the reviewers are taking so much interest in improving the paper for the readers of the journal, some of the questions that they are focusing on may be superfluous in the light of accreditations that this hair growth formulation has already achieved. And therefore, we request you to take a more holistic view and complete the process at your earliest, so that the readers of his journal can benefit from the information published in this paper.

Thank you for your consideration.

Sincerely,

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

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

Conflict of Interests: The authors have been awarded a patent from the United States Patent & Trademark Office (USPTO) & from the Indian Patent & Trademark Office, for the invented hair formulations, used in this study

Funding: Nil.

Key words: Alopecia; Hair Loss; Growth Factors; Hair growth; QR 678 Neo hair growth factor formulation; QR 678

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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. The formulation is injected in the intradermal layer of the skin of the scalp and may prevent hair loss and stimulates hair growth. The first clinical trial in humans demonstrated a significant reduction in hair loss in 83% of the patients using the 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. Seventy-five percent of the patients believed that the injections reduced or stabilized the hair loss. A positive effect on the hair loss was seen in post-hair transplant patients. At one year, a statistically significant increase in

total hair count (P=0.002) was seen. The treatment was well tolerated. [7,8]

This formulation was also compared with the results of Platelet Rich Plasma (PRP), in a split head human trial & results were almost 80% better with the QR 678® treatment, when compared with PRP. [9]

Considering that this formulation has already demonstrated encouraging clinical results in a human trial & been awarded a composition patent by the United States Patent & Trademark Office (USPTO) and Indian Patent & Trademark Office (IPTO), we thought it pertinent & necessary to publish the preceding animal trial & cytotoxicity data, from the data on file.

MATERIALS AND METHODS:

Before conducting the aforementioned pilot human study[7,8], the QR 678 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 documents the same animal study mentioned above.

Kapoor and Shome have also introduced a new variation of the same formulation called as QR678 Neo. It is a plant derivative, consisting of biomimetic peptides including Sh-Polypeptide 9 [bio-mimicking Vascular endothelial growth factor (VEGF)], Sh-Polypeptide 1 [bio-mimicking Basic Fibroblast growth factor], Sh-Oligopeptide 2 [bio-mimicking Insulin like growth factor (IGF-1)], Copper tripeptide-1, Sh-Polypeptide 3 [bio-mimicking Keratinocyte growth factor (KGF-1)] Sh-Oligopeptide 4 (bio-mimicking Thymosin Beta-4 (Thymosin β4)]and vitamins, minerals and amino acids.[10] Although it has been proved that plant derivatives are biomimetic polypeptides of the growth factors and changing the source does not affect

the efficacy of the product,[11,12] we wanted to confirm the same through our study. A similar animal & cytotoxicity trial was carried out using QR678 Neo and it was interesting to note that the results obtained were similar in both QR678® and QR 678 Neo.

Reported therapeutically acceptable ranges of the growth factors used in QR 678 & QR 678 neo 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 (Human Polypeptide-3) – 0.01mg/L – 100 mg/L
vi. Thymosin β4 – 0.005mg/L – 100 mg/L

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 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).

Sample Size :

The sample size was calculated through the standardized protocol of a priori power analysis using the G* Power Software (Faul, Erdfelder, Lang and Buchner, 2007). The effect size was duly adjusted at 0.5 during computation of power analysis. The power analysis was based on 1mm hair growth length.The sample size that was derived was a minimum of 15 for In vivo Study Arm 1(Stimulation of hair growth by representative growth factors & biomimetic peptides in mammals) and 50 for In vivo Study Arm 2.

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, 15 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. In mice with an agouti coat, such as C3H/HeJ mice, hair clipping allows distinction easily between anagen and telogen phases, due to the fact that in agouti mice hair follicles switch from light coloured pheomelanin to dark coloured eumelanin production during anagen. A black band of anagen hairs enables visualization and monitoring of the timing of the anagen spreading wave. These mice therefore represent an excellent model to study the hair cycle.

When the hair is in telogen, it can grow again only when the hair root enters the anagen phase on its own again after a specific interval of time. So, if a particular substance or molecule needs to demonstrate that it actually stimulates hair growth, it has to be administered in the telogen phase, so that it can stimulate the follicle to enter anagen growth phase, thus shortening telogen & causing premature entry of the resting follicles into anagen phase. Usually, the dorsal part of the animals is used for the testing.[13] As a standard, hair follicles from the dorsal skin from postnatal days 1 to day 12 are typically used to represent anagen, from day 17 to represent catagen, and from day 21 to represent telogen.

Most importantly, the 3 stages of the hair cycle are well characterized in the mouse: anagen (growing phase), catagen & telogen (resting phase). The first two cycles of the mouse hair follicle are synchronized; that is all hair are in one stage of development. The mouse hair cycle is short, lasting for 3 weeks & the hair follicles can be examined

at specific time points in the cycle. [14]

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. The mice were kept under observation & evaluated at days 1, 3, 7, 14, 28. (**Table 1**)

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 rat pups 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 intraperitoneal 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:

Grade Degree of alopecia

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.

Reported oral toxicity here is defined as the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals.

i) In vitro, human keratinocyte cell (**figure 1**) as well as human fibroblast cell (**figure 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 (**figure 3**) & human fibroblast cell (**figure 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 (**figure 5**) & human fibroblast cell (**figure 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 (**figure 7**) & human fibroblast cell (**figure 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 (**figure 9**) & human fibroblast cell: (**figure 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 (**figure 11**) & human fibroblast cell (**figure 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 2**

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 (Figure 13), follicles located away from the injection were dystrophic & nonfunctional (disruption of the integrity of inner and outer root sheaths, & disrupted hair shafts) (Figure 14). 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]

Various non-surgical options for treating hair loss are available. Oral finasteride and topical minoxidil alone or in combination have shown adequate results. However side effects with their long term use are not uncommon. Loss of libido with finisteride use . and headaches, tachycardia as well as increase in body hair with the use of minoxidil have been frequently noted. [15,16]

Platelet-rich plasma (PRP) has also been studied as a method for hair regrowth in male pattern hair loss. Literature review suggests that, injections of PRP are safe and feasible treatment option for androgenetic alopecia, with high overall patient satisfaction.[17-21]

Multiple trials have also been published highlighting the role PRP on hair growth. However, methodological inadequacy has been noted in most of the studies.[22] Most noteworthy shortcoming is the lack of standardized device and protocols that define the preferred method for producing PRP. As mentioned by Lynch and Bashir, PRP is usually prepared on a per-patient basis. Approximately 8 to 60 ml of fresh venous blood is drawn, collected and centrifuged, leading to the separation of the erythrocytes from lighter plasma with a buffy coat at the interface. The plasma and buffy coat are

then aspirated and mixed.[23] Other drawbacks include lack of a reference protocol mentioning the frequency of applications and the amount of PRP to be injected, lack of detailed reports in patients' characteristics heterogeneity in mode of application, lack of controls, small sample size, and used statistical methods.[22] 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 [24] 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 mice, after intradermal injection of various concentrations of the growth factors and peptides of the formulation. The mice were kept under observation & evaluated at days 1, 3, 7, 14, 28. 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) and the same was used in the human trial subsequently.[7] In human trials, our injection protocol was for 8 sessions of treatment, given at 3-4 weekly intervals.[7]

In a study conducted by Shome et al, comparing the efficacy of QR678 with PRP in Male Androgenetic Alopecia, a reduction in hair fall (i.e.: pull test became negative i.e.: number of hair pulled is 3 or less) was noted in all the patients of QR678 group by the end of 8th session (6months) whereas; the hair fall was reduced (pull test negative) in just 50% in PRP group. The results were maintained in QR678 group at 1 year follow-up although the number of hair pulled was increased in PRP group at the end of 1 year.[9] Also, on videomicroscopic assessment(evaluating the hair density, terminal hair density, vellus hair density, and shaft diameter) showed significant improvement ($p<0.005$) with QR678 group, wereas the results were not significant with PRP group. On global photographic assessment and patient self assessment also, the results of QR678 were significant and superior to PRP. Side effects like itchy scalp, unsteadiness during injection and increase in hair fall were also negligible in QR678 group as opposed to PRP group.[9]

In our study, 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. Various newer therapies have emerged in the domain such as dutasteride microinjections, topical finasteride, Setipiprant, micronedling, Simvastatin etc. However, on literature review, no clinical trials were encountered and the limited number of studies lacks enough potential to be termed as

efficacious for acceptable treatment outcome for post chemotherapeutic drug induced alopecia.[25]

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 and 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 and 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.[26,27] KGF is highly capable of counteracting chemotherapy induced alopecia & it is one of the components of our formulation.[28] 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.[29-31] Thymosin B4 promotes hair growth in various rat

& mice models, including a transgenic thymosin B4 overexpressing mouse, by influencing follicle stem cell growth, migration, differentiation, & protease production.[32] 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.[33] 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.[34]

In summary, we publish the results of the pre-clinical data on file, demonstrating the safety and efficacy of intrdermal QR 678® and QR 678 Neo hair growth formulations. The findings of cellular assays and animal studies suggest that this QR 678® and QR 678 Neo formulations are safe and efficacious in treating hair loss in mammals. The 2nd arm which was introduced in the study to check the effect of QR678® and QR 678 Neo on cytosine arabinoside induced hair fall (chemotherapy-induced secondary alopecia) also showed encouraging results. Our evaluation of the QR678 Neo, which is a plant derivative, consisting of biomimetic peptides, showed similar safety and efficacy to the QR 678®. Although it has been earlier proven that plant derivatives are biomimetic polypeptides of the growth factors and changing the source does not affect the efficacy of the product, we wanted to confirm the same through our study.

LIMITATIONS AND FUTURE SCOPE:

The limitation of the study was the small sample size. This was a pre-clinical study, demonstrating the safety & efficacy of the hair formulation, which we call the QR 678® hair growth factor injections. One arm with cytosine arabinoside induced hair fall was included in the animal trials to check if the QR 678 ® and QR 678 Neo could

also work in chemotherapy-induced secondary alopecia in addition to androgen-induced alopecia. Based on the promising and encouraging results of the current study, a human trial was subsequently conducted and yielded good results.[7] Also, future studies are warranted showing the effect of QR678® and QR 678 Neo on disorders affecting hair fall like inflammatory conditions of the scalp including, but not limited to atopic dermatitis and autoimmune hair disorders like Alopecia areata. The evaluation of the QR 678® formulation in androgenetic alopecia has already been done in a human trial. A separate human trial to evaluate the results of QR 678® and QR 678 Neo hair formulations in a trial in human patients with secondary alopecia, post cancer chemotherapy is warranted in the future.

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TABLE LEGENDS:

Table 1: Solutions with varied concentrations of growth factors injected in mice in in vivo study 1.

Table 2: Toxicity data of the growth factors used in the formulations.

Table 3: Hair growth response seen in mice in in vivo study 1.

Table 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.

FIGURE LEGENDS:

Figure 1: Cytotoxic effect of bFGF on Human keratinocyte cells in vitro

Figure 2: Cytotoxic effect of bFGF on Human fibroblast cells in vitro

Figure 3: Cytotoxic effect of IGF-1 on Human keratinocyte cells in vitro

Figure 4: Cytotoxic effect of IGF-1 on Human fibroblast cells in vitro

Figure 5: Cytotoxic effect of KGF on Human keratinocyte cells in vitro

Figure 6: Cytotoxic effect of KGF on Human fibroblast cell in vitro

Figure 7: Cytotoxic effect of VeGF on Human keratinocyte cells in vitro

Figure 8: Cytotoxic effect of VeGF on Human fibroblast cell in vitro

Figure 9: Cytotoxic effect of Thymosin B4 on Human keratinocyte cells in vitro

Figure 10: Cytotoxic effect of Thymosin B4 on Human fibroblast cells in vitro

Figure 11: Cytotoxic effect of Cu GHK on Human keratinocyte cells in vitro

Figure 12: Cytotoxic effect of Cu- GHK on Human fibroblast cells in vitro

Figure 13: Histological section of functional anagen hair follicles ,stained with

hematoxylin and eosin (H&E), observed at the site of injection of the hair formulation. Scale bar: 100µm.

Figure 14: Histological section of dystrophic & nonfunctional, stained with hematoxylin and eosin(H&E),observed at the site of injection of the hair formulation. Scale bar: 100µm.

Table

	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

1mg/L means 1 ppm

Table 1

	Toxicity Data	**Reference**
IGF-1	EDso ≤10.0ng/ml using mouse Balb/3T3 cells	Ref 1 (IGF-1)
	Endotoxin level: <0.10 EU per 1µg 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 threashold. Mouse Intraperitoneal, LD50 :154mg/kg	Ref 2 (bFGF)
	Endotoxin Level: <0.10 per 1 µg of the protein by LAL method	Ref 2-1 (bFGF)
KGF	Endotoxin Level: <0.10 per µg of the protein by LAL method	Ref. 3(KGF)
VEGFA	Endotoxin Level: <0.01 per 1 µg of the protein by LAL method	Ref 4(VEGFA) Ref 4-1 (VEGFA)
TMSB4	Endotoxin Level: <0.1ng/ µg of the protein (<1EU/ µg)	Ref 5(TMSB4) Ref 5-1 (TMSB4)
Noggin	Endotoxin Level: <0.10 per 1 µg of the protein by 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

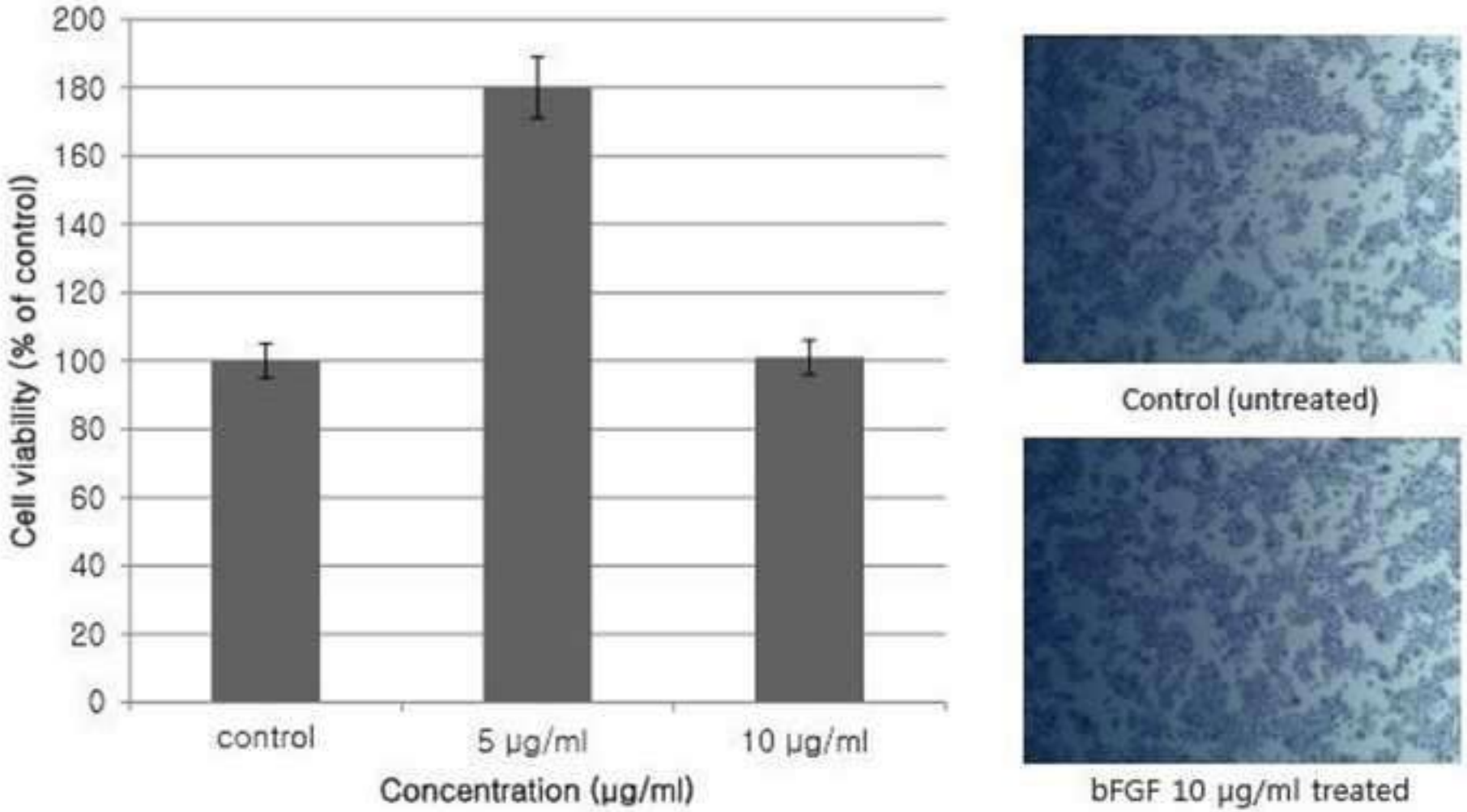
Composition	Number of animals growing hair	Day of onset	Area of hair growth around the injection site
Solution 1	2/5	10	< 1 cm diameter
Solution 2	3/5	10	> 1 cm diameter
Solution 3	5/5	10	> 1 cm diameter
Solution 4	4/5	10	> 1 cm diameter
Solution 5	4/5	10	>1cm diameter
Solution 6(saline control)	0	NA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

Table 3

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	1.91

Table 4

Figure 1



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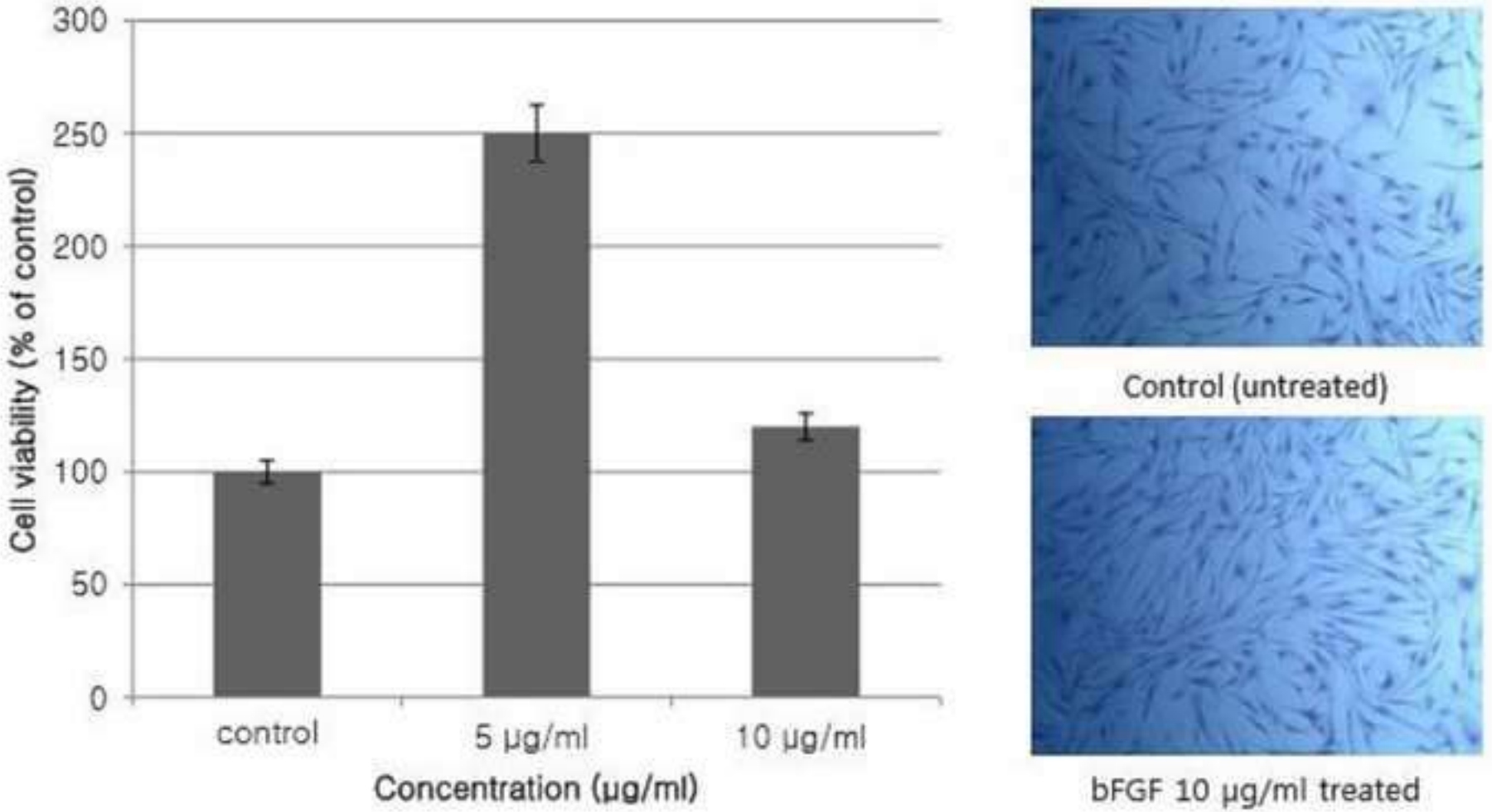

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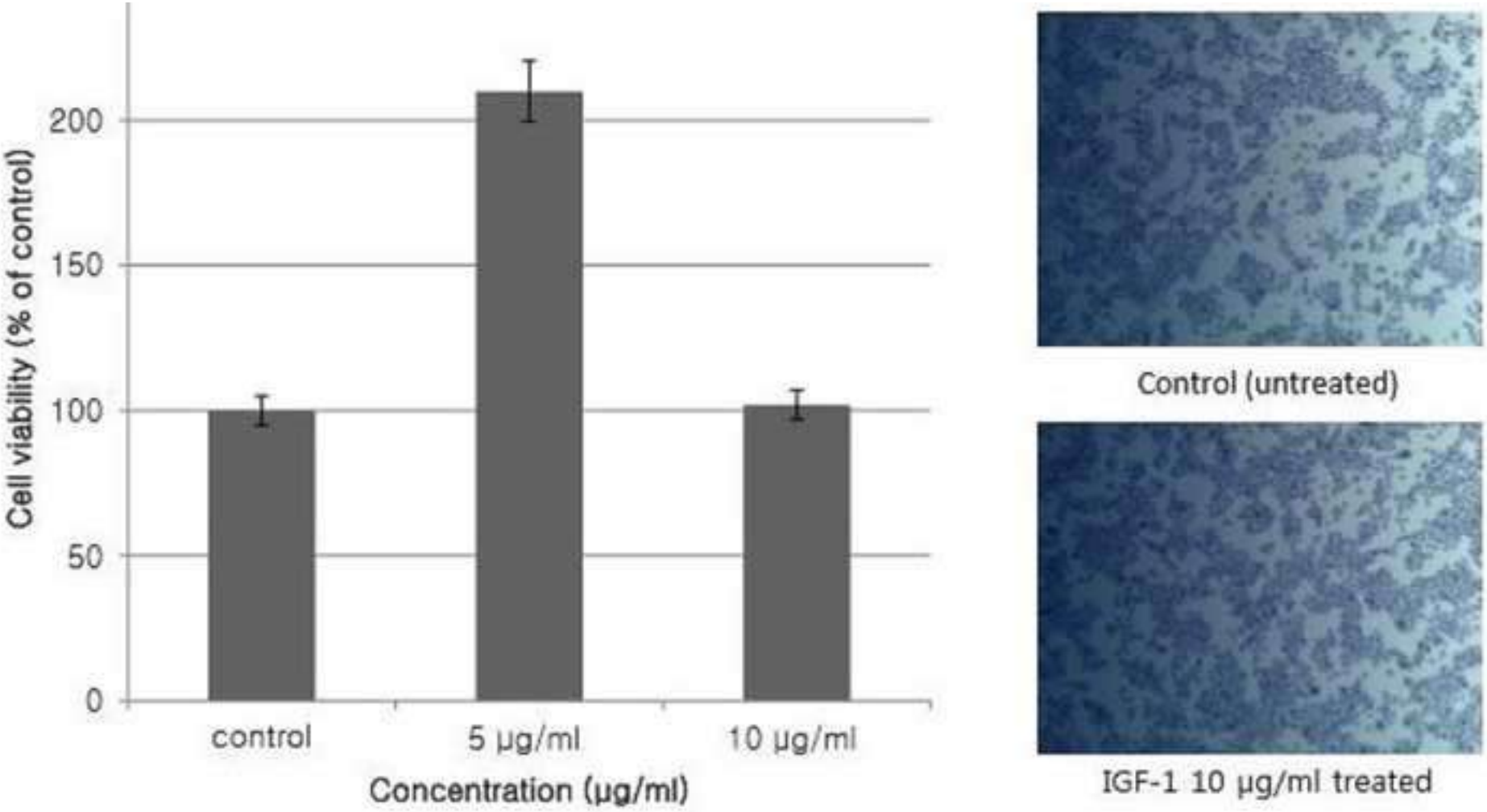

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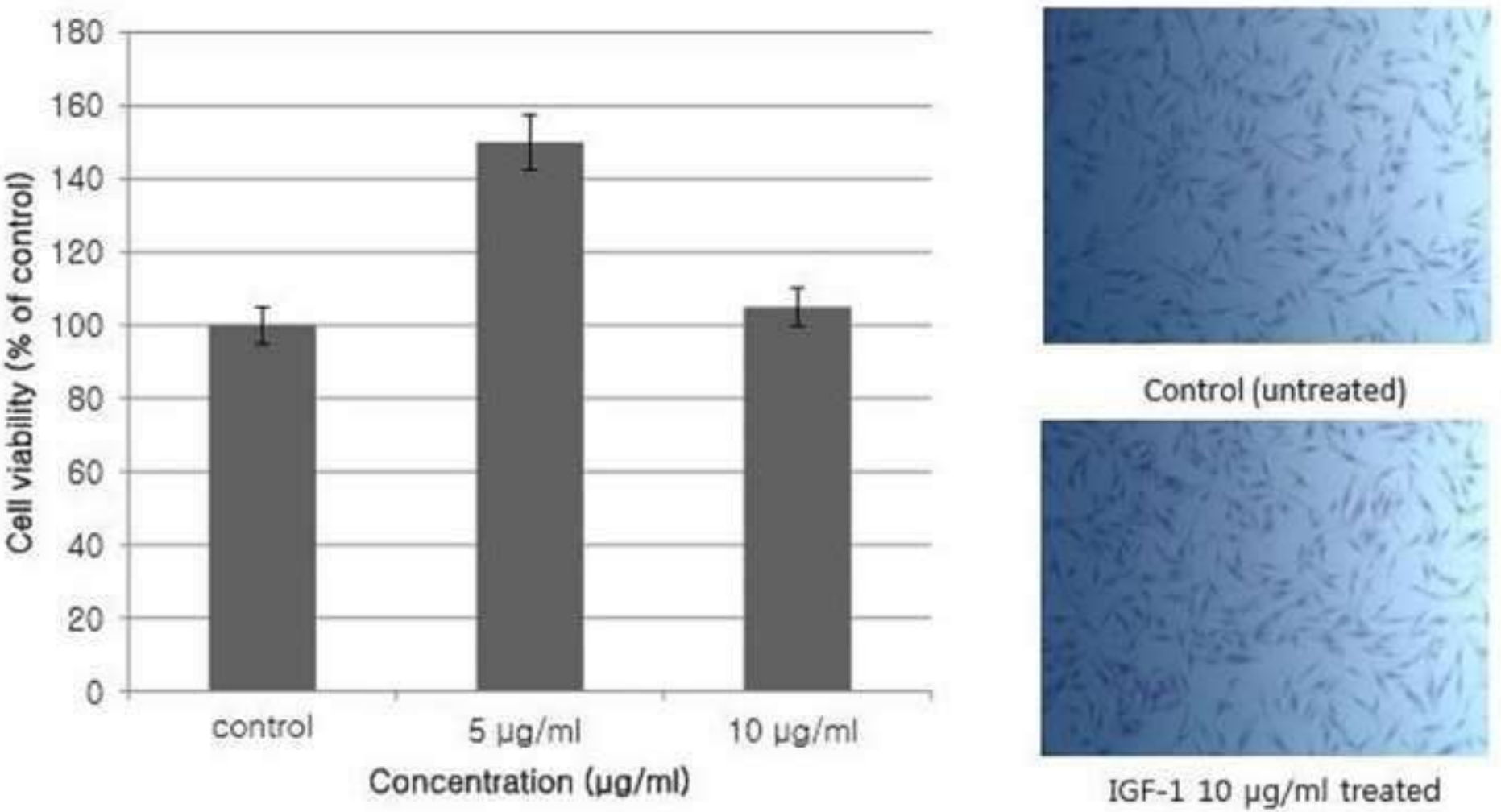

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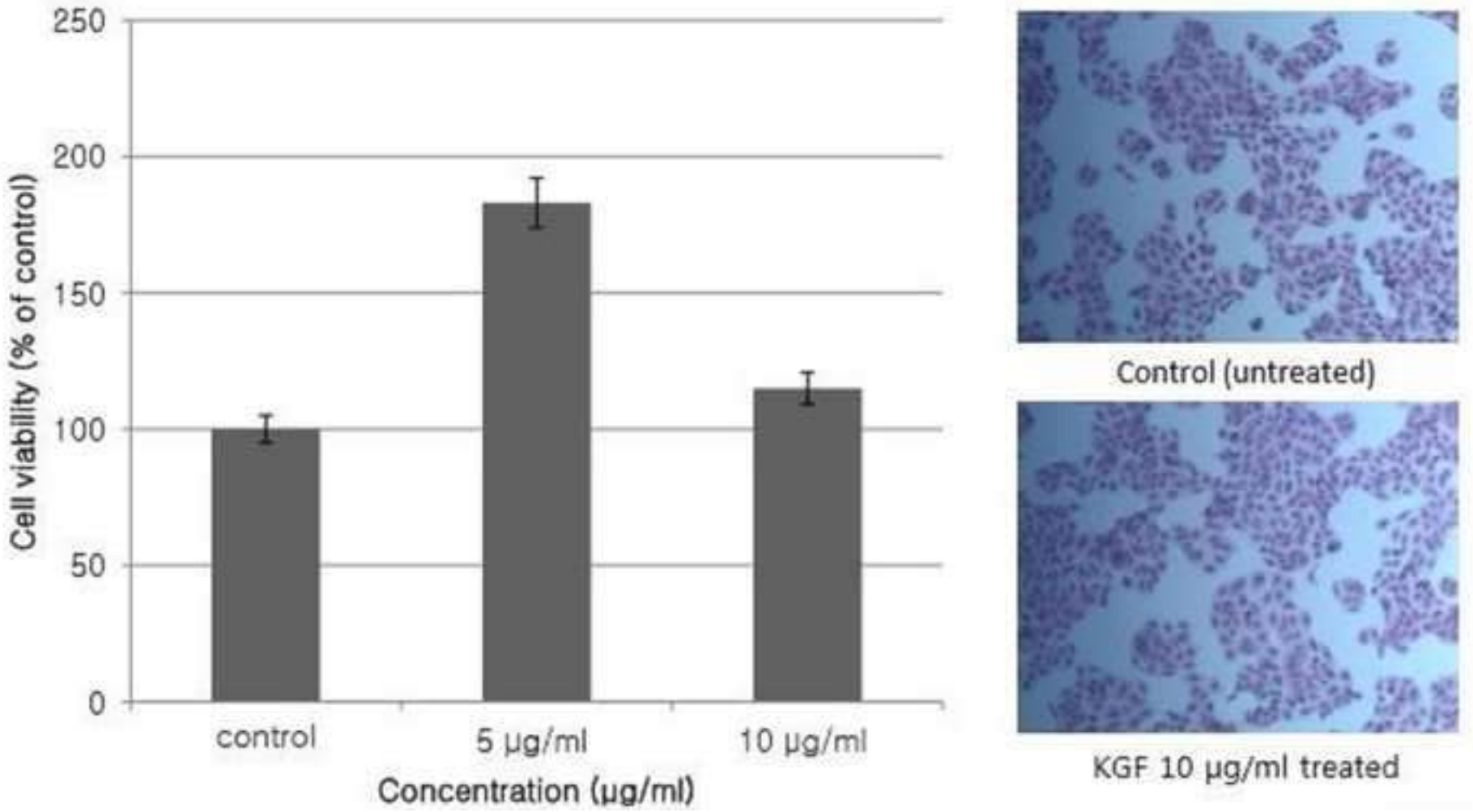

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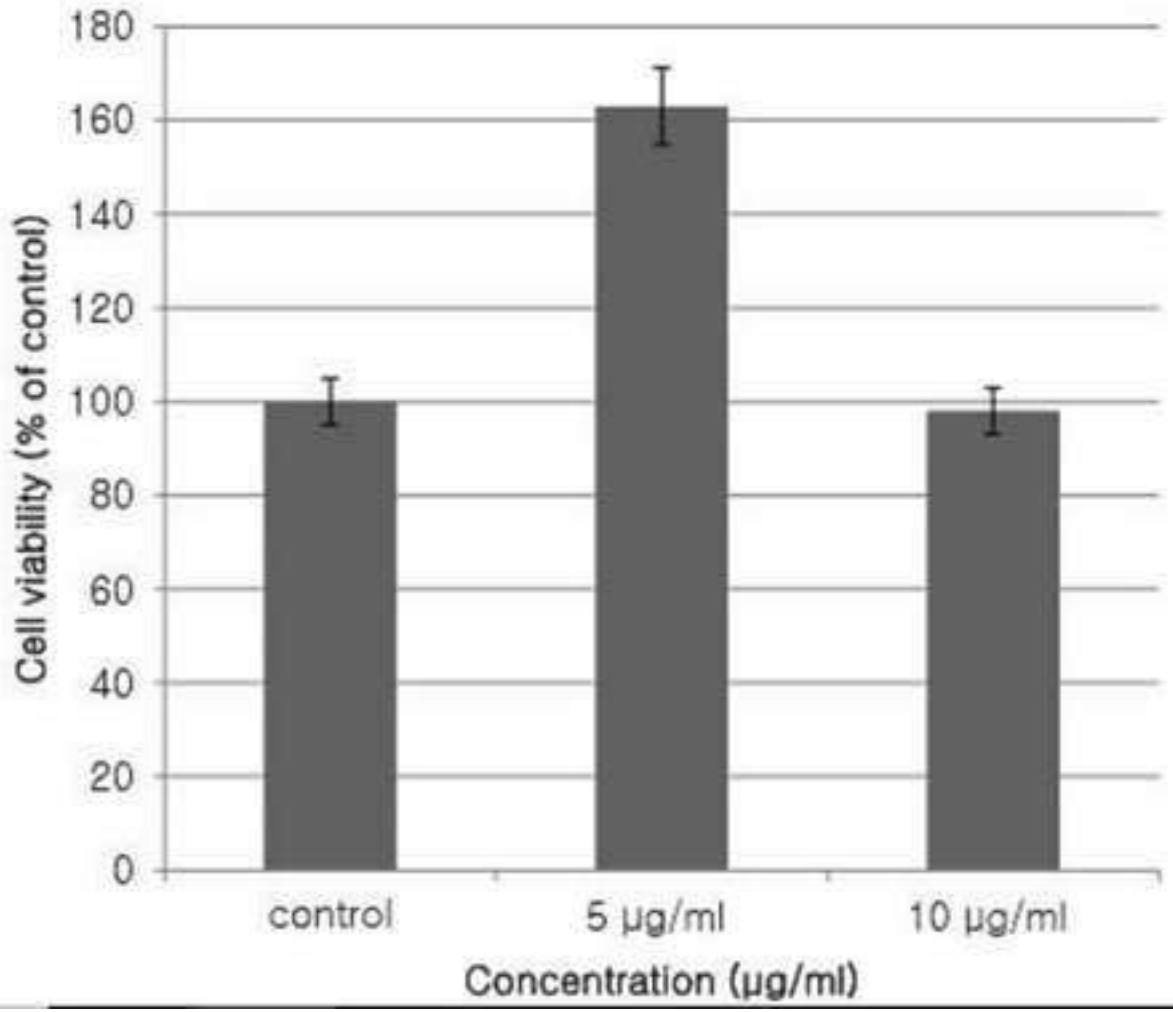
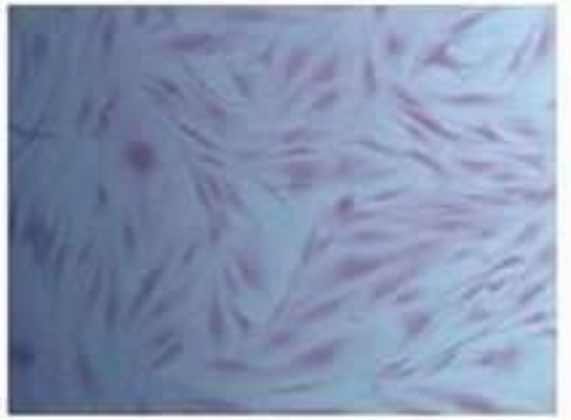
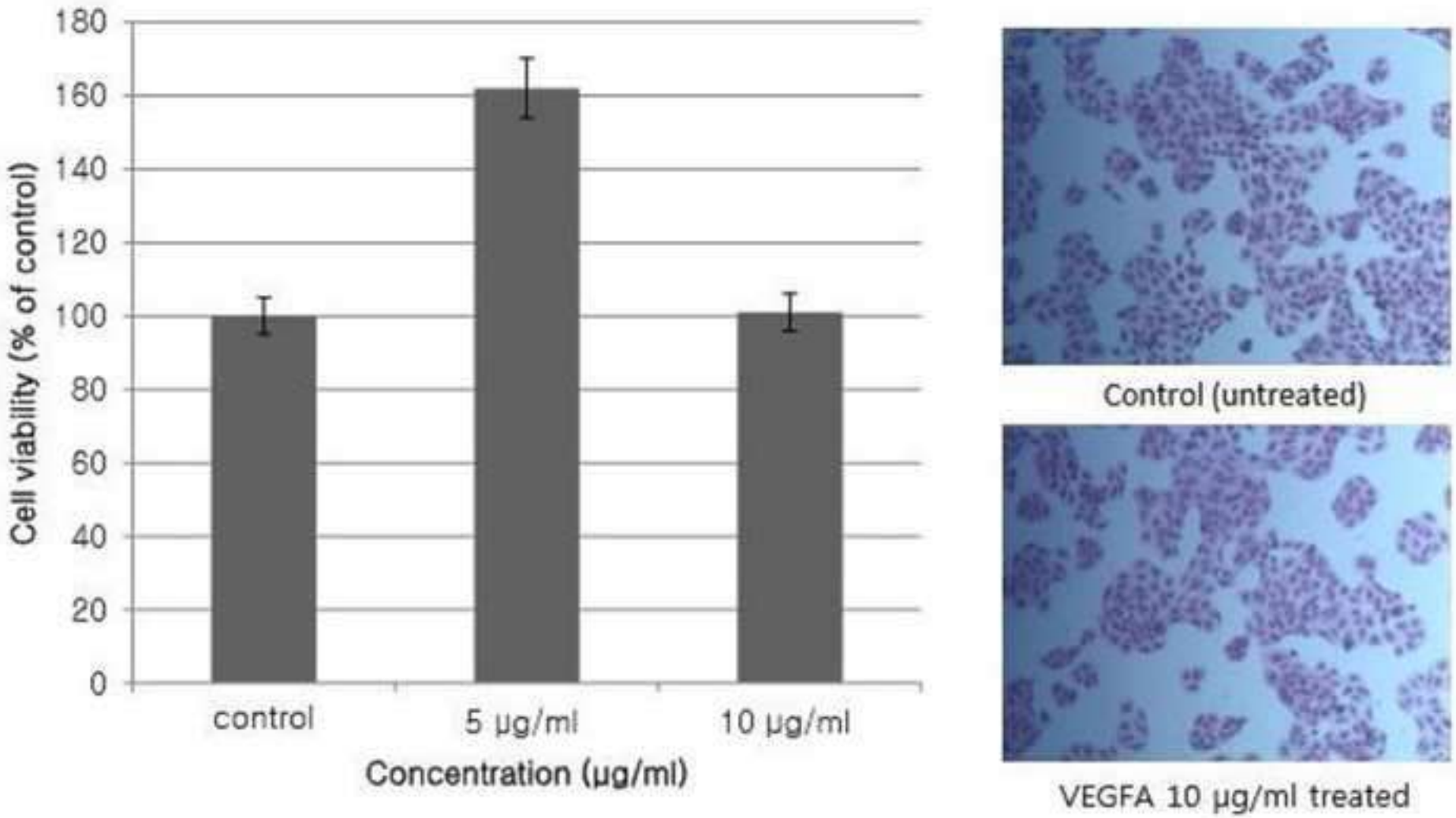




Control (untreated)

KGF 10 µg/ml treated

Figure 7



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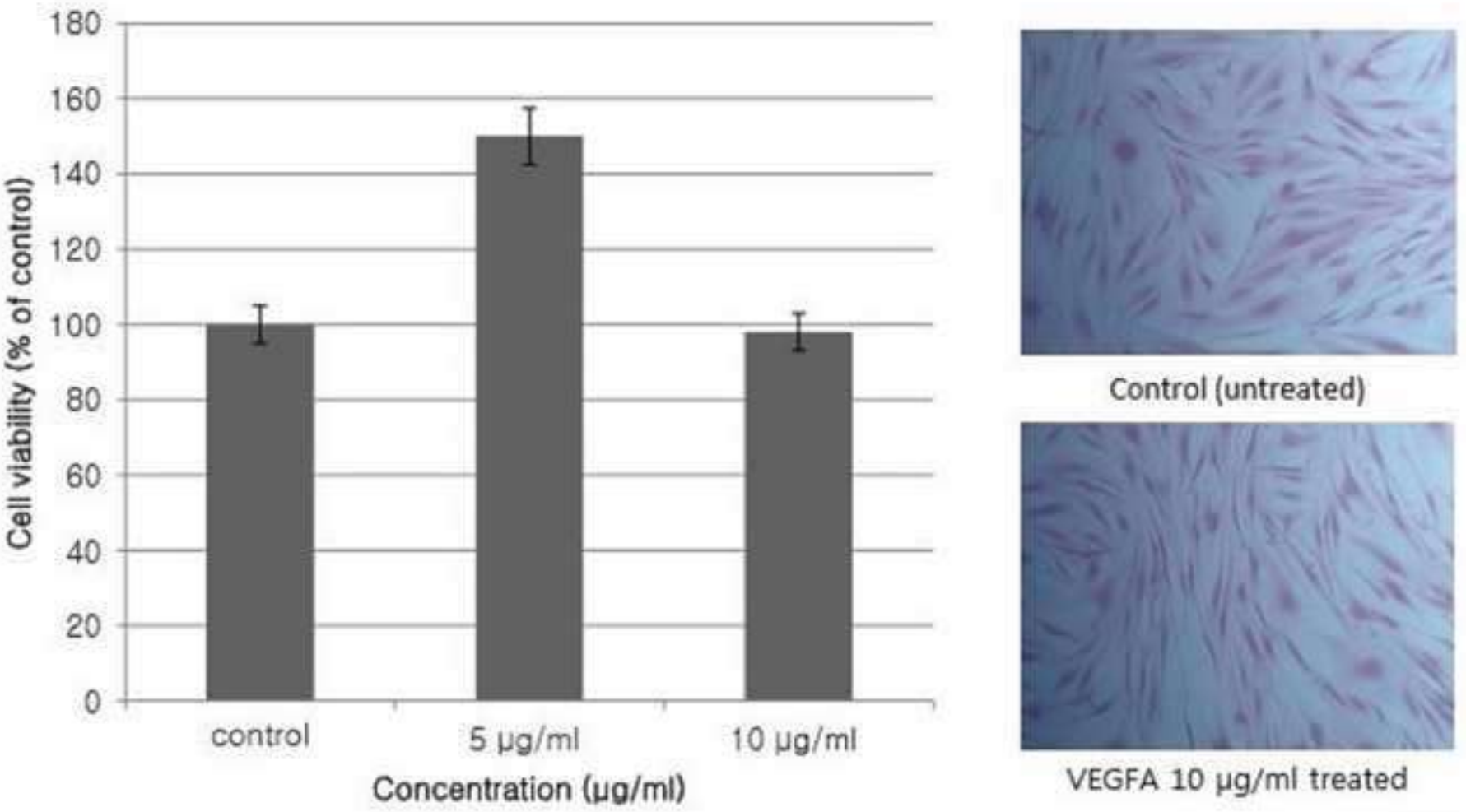

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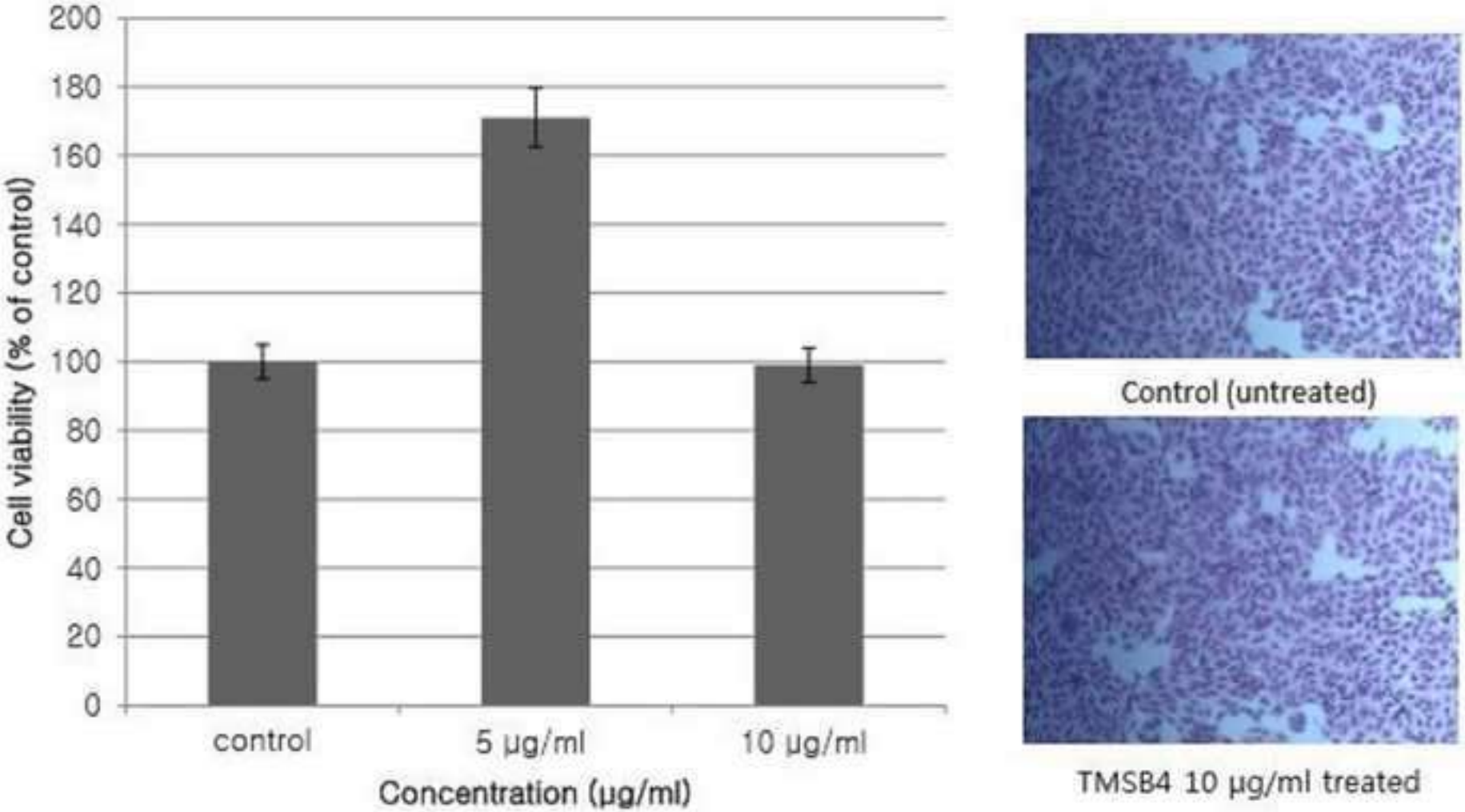

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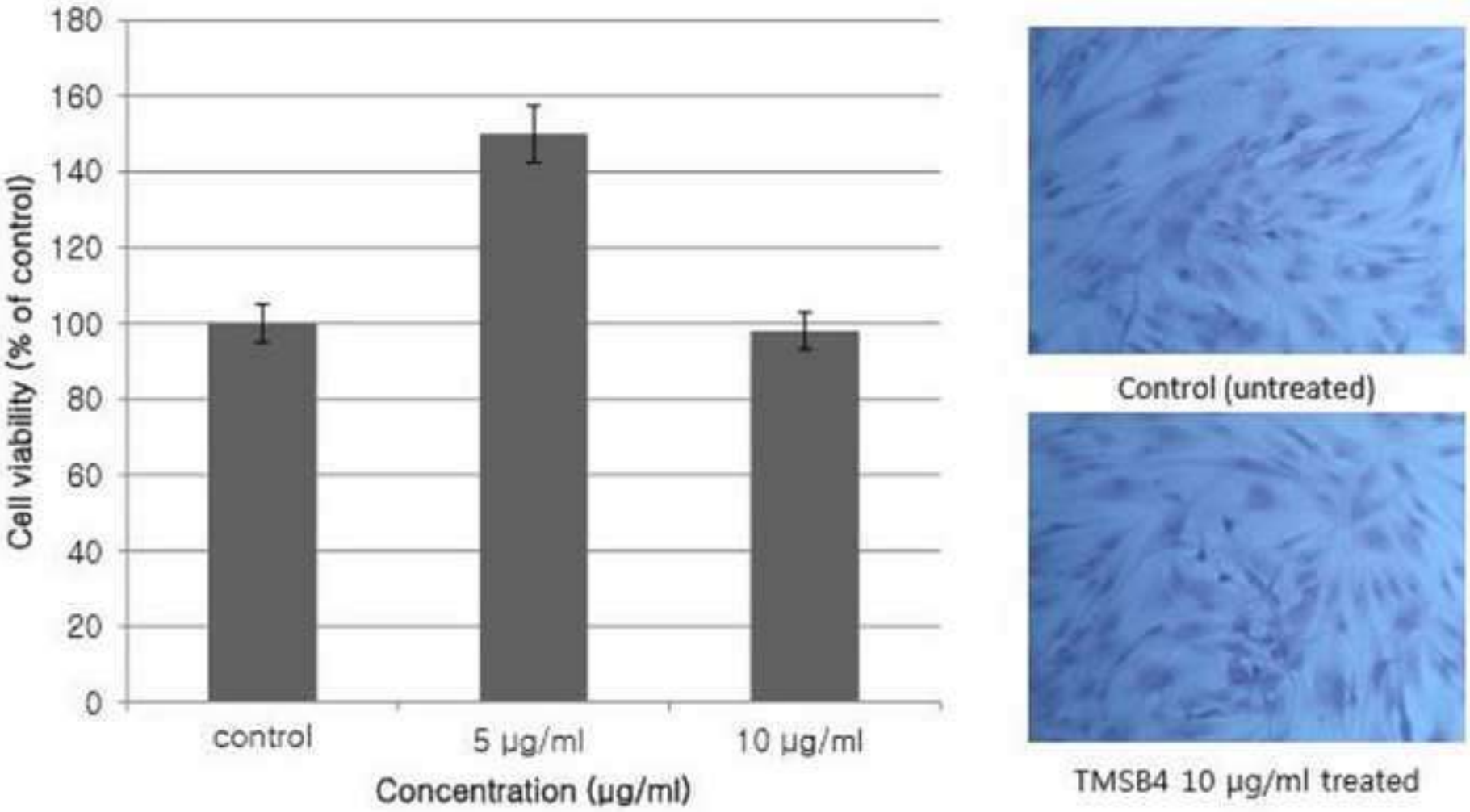

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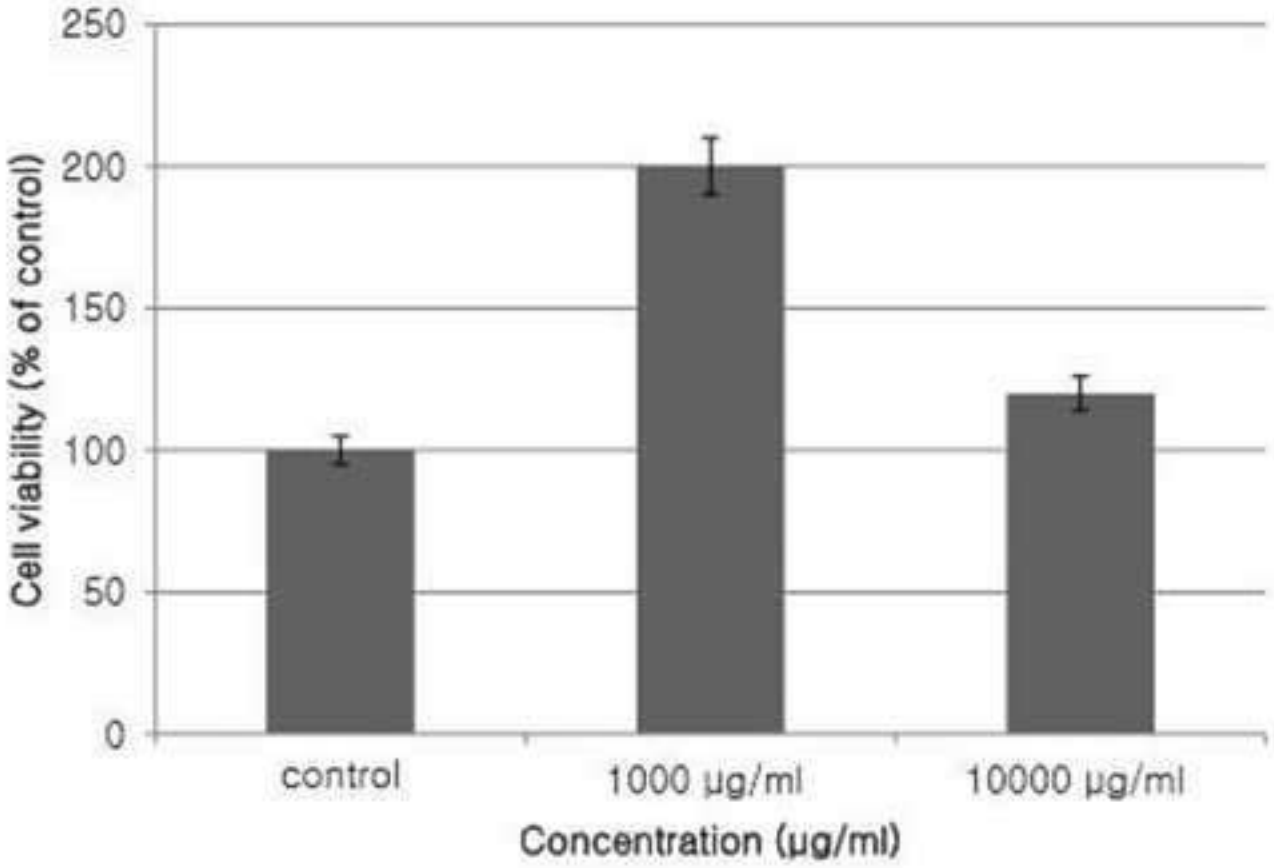

Control (untreated)

Cu-GHK 10,000 µg/ml treated

Figure 12

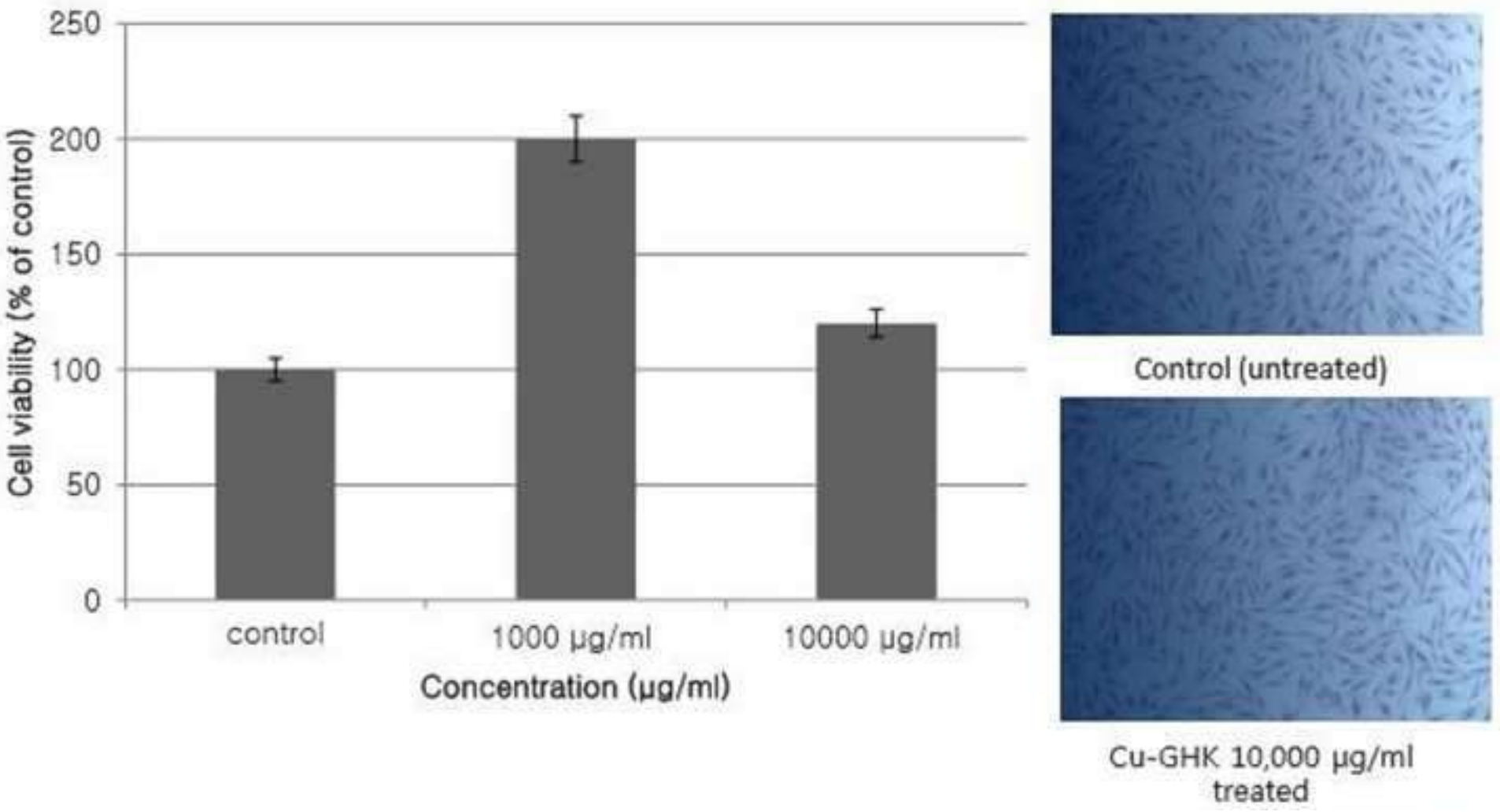

Figure 13

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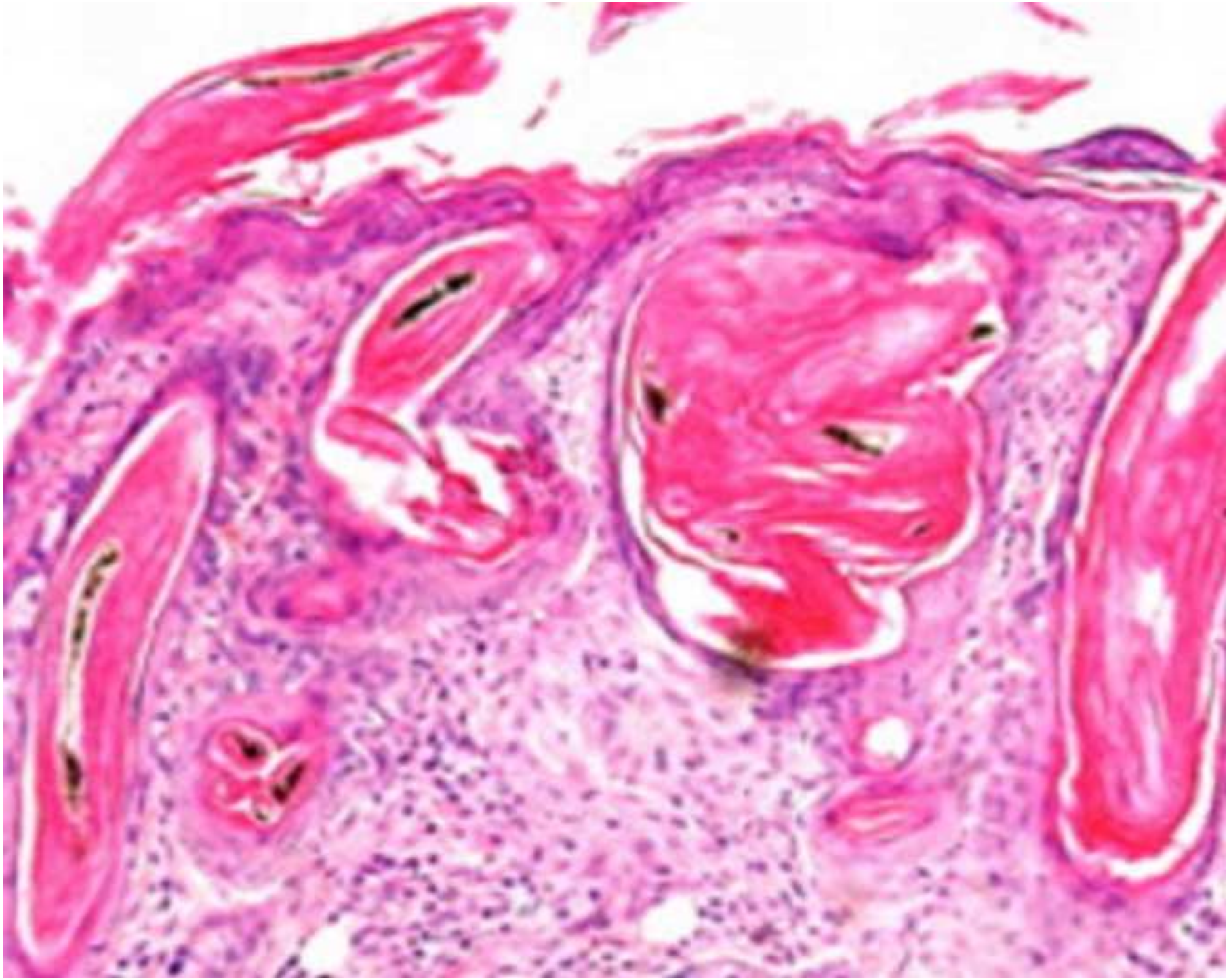

Figure 14

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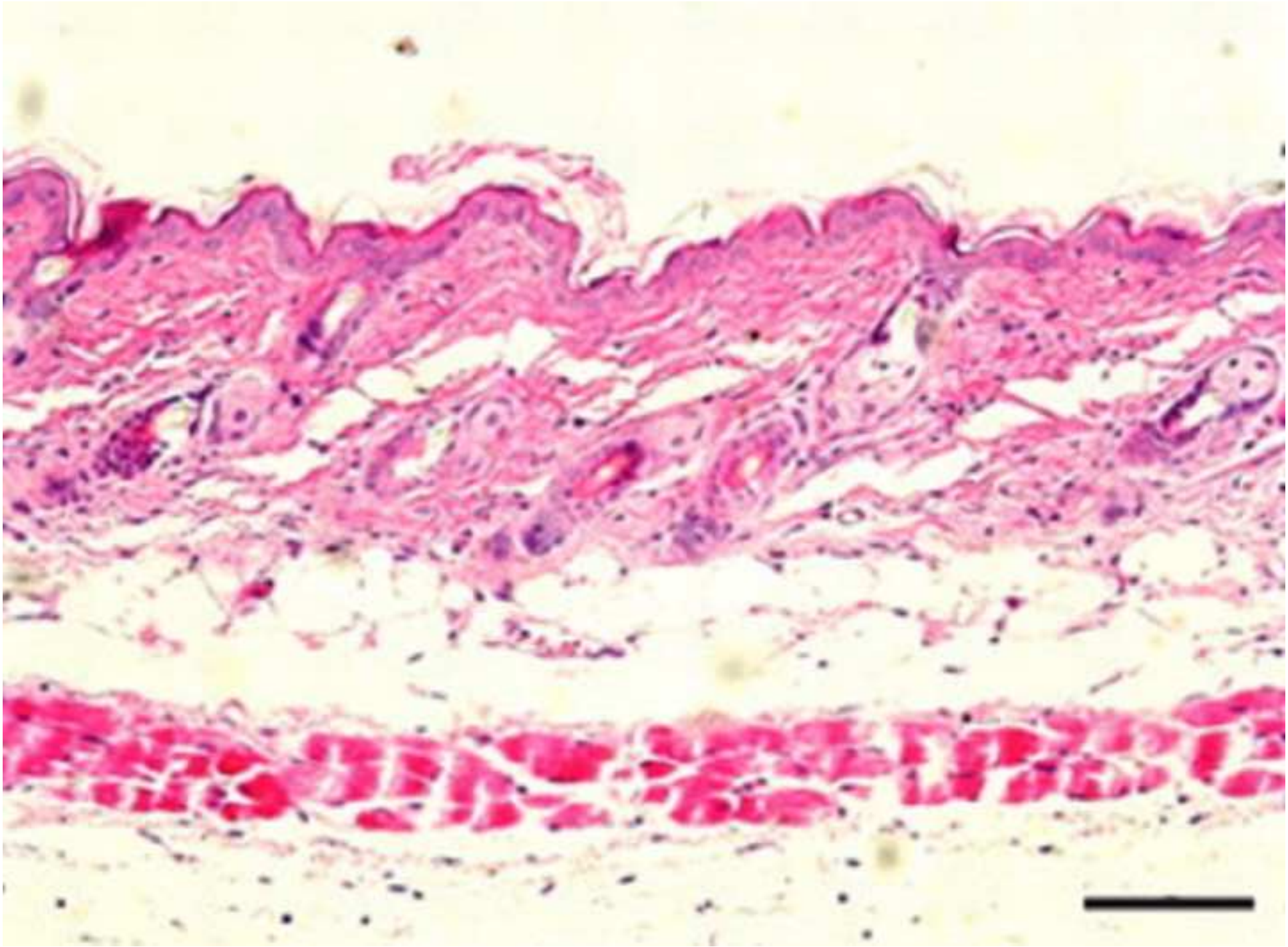

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