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Synergism between Tocotrienol and i-PRF in HFSCs proliferation: an in-vitro study

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Abstract

Introduction: Androgenic Alopecia (AGA) is an early onset of hair loss found in both males and females. With an increase in stress and hormonal imbalances, the need for a better and faster alternative to today's medicine for alopecia is increasing. Adding an adjunct like Aloe Vera to the gold standard platelet concentrate in the treatment of alopecia may boost hair growth. To find an optimum concentration and evaluate the synergism between Aloe Vera and injectable platelet-rich fibrin (i-PRF) is the aim of this in-vitro study.

Materials & Method: Hair follicles from an individual suffering from AGA were prepared and stored in an incubator. Stem cell characterization was done and stem cells were multiplied. Hair follicle-derived stem cells (HFSCs) were subjected to different concentrations of Aloe Vera, i-PRF, and i-PRF with Aloe Vera in different Petri dishes.

Result: Aloe Vera group shows an increase in HFSCs proliferation up to a certain concentration, after that it shows cell senescence. I-PRF on other hand shows a direct relation between concentration and cell proliferation. The combination group at a particular concentration shows double the cell proliferation than the rest of all.

Conclusion: Alopecia and its treatment are in a wide range and have evolved with time. No single treatment modality can be sufficient to treat a disease at its best. Adjunct therapy when incorporated into the standard treatment results in faster and better hair growth. Also, the optimization of the concentration of any product is of great value.

Key Points

Hair loss requires an upgradation in its method of treatment. With inclusion of an adjunct to the mainstream therapy will manifest into better results on hair growth.

Introduction

Androgenetic alopecia (AGA) is a type of hair loss that follows a predetermined pattern and is characterized by progressive thinning of scalp hair. It is hereditary and androgen-dependent, and in some men, onset can occur during puberty. Alopecia can significantly affect a person's self-esteem and confidence. The incidence of AGA is least seen in Aboriginal Americans and Africans/African-Americans, while the Asian population shows different clinical and genetic characteristics compared to the European population. Although AGA may not be life-threatening, it can be emotionally strenuous and lead to depression, particularly in women [1].

Various factors can affect hair growth, leading to alopecia. Inflammation and *oxidative stress* can contribute to hair loss. Exposure to ultraviolet light, smoking, pollutants, and poor nutrition can all

produce reactive oxygen species. Genetics also plays an important role in male AGA, with numerous studies showing linkage to the 'X' chromosome, specifically the androgen receptor/EDAR2 locus [2].

The standard treatment for alopecia is a combination of 1 mg oral finasteride and 5% topical minoxidil, which can be used separately as well [1]. However, various treatment modalities have emerged to overcome the adverse drug reactions (ADRs) of minoxidil and finasteride. These include platelet-rich plasma (PRP), injectable cytokines, nutraceuticals, and low-level laser therapy (LLLT) [2].

Platelet concentrates have been known for their regenerative capacity for over three decades, releasing a high number of growth factors with multidisciplinary uses in regenerative dentistry, aesthetic medicine, maxillofacial surgery, orthopedic surgery, and more. Although PRP has shown an immense array of practice, various anti-coagulants and bovine thrombin have been known to show tissue regenerative suppression activity. *Platelet-rich fibrin (PRF)* has been developed to overcome this disadvantage and becomes the first autologous blood-derived growth factor without any anti-coagulants [3].

PRF in common is a specific form of matrix meshwork and hence, does not have the required flowability. *Injectable PRF* is methodically made by using a centrifuge at a slow rate, which typically allows PRF to stay in an injectable form [4]. For this reason, i-PRF has been used in this study instead of PRP or PRF, as i-PRF has been proven to yield better results than PRP [4].

Incorporating an adjunct to regular treatment may enhance cell growth and reduce the need for multiple sittings. *Aloe vera* is an Ayurvedic medicine with immense medicinal value. The gel-like tissue from the leaves of Aloe Vera is popular in cosmetic and therapeutic uses [5]. *Tocotrienol* is a component of Aloe Vera and has anti-inflammatory properties that help in reducing oxidative stress [6].

This study aims to evaluate the synergistic effect of tocotrienol when used as an adjunct to i-PRF to determine the proliferative potential of *hair follicle-derived stem cells (HFSC)* in an in-vitro setup.

Material & Method

The Regenerative Medicine Laboratory (RML) at our institute conducted an in-vitro study in March 2022 to evaluate the potential of hair follicle-derived stem cells (HFSC) in patients with androgenetic alopecia (AGA). The study was approved by the institutional stem cell research committee, scientific committee, institutional ethics committee, and review board (DPU/484/17/2021).

Patient selection criteria included individuals between the ages of 18–35 with a diagnosis of AGA and no systemic diseases such as hemophilia, diabetes, epilepsy, etc. Before the procedure, the patient was given information about the pre-operative preparation and operative procedure and provided valid informed written consent in a language they understood. An experienced hair transplant surgeon harvested 30 hair follicular units from the patient, and post-operative wound care instructions were provided.

I. Isolation and culture of human hair follicle stem cells (HFSCs)

After harvesting, the hair follicles were transported to RML in a phosphate-buffered solution (PBS) and processed by a research scientist (AK). The hair follicles were then traumatized using a No. 15 blade and transferred to a petri dish. After segregating them, the hair follicles were covered with fetal bovine serum (FBS) and incubated at 37°C with 5% CO2 for 24 hours. The explant culture was then maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 20% FBS and antibiotic-antimycotic (AA) solution. The culture medium was changed every 2–3 days, and the cell outgrowth was monitored using an inverted phase-contrast microscope (Olympus CKX53, Japan). Once enough outgrown cells were obtained at 70–80% confluence, they were detached using 0.25% trypsin-ethylene diamine tetra acetic acid (EDTA) and transferred to a 75 cm2 flask (Fig. 1).

II. Characterization using flow cytometry

Stem cell isolation was characterized using flow cytometry analysis with CD73, CD90, CD105, CD34, CD45, and HLA-DR antibodies (all from eBiosciences, USA). The cells were analyzed using Attune NxT flow cytometry (Thermo Scientific, USA). After stem cells were isolated, their cytotoxicity at different concentrations of Aloe Vera, i-PRF, and i-PRF with Aloe Vera was checked using the water-soluble dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Cell viability and proliferation were ascertained using the formazan dye at 24 and 48 hours (Fig. 2).

Result

The stem cells extracted from human hair follicles were isolated and cultured in different Petri dishes (Fig. 3). Various concentrations of i-PRF and Aloe Vera were added to the Petri dishes, and the proliferation of HFSCs was evaluated after 24 and 48 hours. The results showed that the HFSCs proliferation varied with different concentrations of i-PRF and Aloe Vera.

At a concentration of 10 µl/ml, Aloe Vera resulted in a growth of 0.025 nm ± 0.01 nm, whereas a concentration of 8.5 µl/ml showed a growth of 0.25 nm ± 0.05 nm after 48 hours. A 0.2 µl/ml concentration resulted in a growth of 0.25 nm ± 0.1 nm. In contrast, the addition of no Aloe Vera resulted in cell proliferation of 0.1 nm ± 0.01 nm.

For i-PRF, a concentration of 10 µl/ml resulted in a growth of 0.3 nm ± 0.05 nm, and a concentration of 8.5 µl/ml resulted in a growth of 0.25 nm ± 0.05 nm. No addition of i-PRF resulted in cell proliferation of 0.1 nm ± 0.01 nm.

When i-PRF was combined with Aloe Vera, a concentration of *10 µl/ml* resulted in a growth of *0.3 nm ± 0.05 nm*, and a concentration of *8.5 µl/ml* resulted in a growth of *0.6 nm ± 0.05 nm*. No addition of i-PRF and Aloe Vera resulted in cell proliferation of 0.1 nm ± 0.01 nm.

Graph 1 shows the comparison of all three groups. A line bar graph evaluates the mean value of Aloe Vera, i-PRF, and i-PRF with Aloe Vera. All three groups showed maximum growth at 8.5 µl/ml

concentration. Significant growth of stem cells was observed only after a concentration of 0.5 µl/ml (Graph 1).

Discussion

Androgenic alopecia is the most prevalent form of hair loss, affecting both males and females who exhibit different patterns of hair loss. While males typically experience a receding hairline, females usually show diffuse hair loss [7]. Addressing hair loss is critical as it has a significant impact on physical appearance and mental well-being [8].

Various treatment options are available in the market to promote hair growth, but optimizing hair regeneration potential could revolutionize hair loss management. However, available treatment options such as minoxidil, furosemide, threads, Botox, and different generations of platelet concentrates have their advantages and disadvantages [9]. Moreover, any pharmacologically prepared drugs can have local and systemic adverse drug reactions [10]. Thus, this study aims to combine two highly potential components for hair growth enhancement.

The results of the HFSCs treated with Aloe Vera showed that a *100% concentration of Aloe Vera induced cell senescence*, as observed in Graph 1. This could be attributed to the *Aloe-emodin* content, a natural anthraquinone present in Aloe Vera [11]. This may explain why a 100% concentration of Aloe Vera leads to more cell senescence than cell proliferation.

The maximum HFSCs proliferation was observed at 8.5 µl/ml and 0.2 µl/ml concentrations of Aloe Vera. Aloe Vera promotes cell proliferation through fibroblast migration, proliferation, and keratinocyte migration, which has also been observed by Teplicki et al. in 2018 [12].

The results of the study on HFSCs with i-PRF showed a direct correlation to cell proliferation. When HFSCs were treated with i-PRF and Aloe Vera, no significant difference in cell growth was observed until the concentration reached 5 µl/ml. At a concentration of 8.5 µl/ml, the cell proliferation rate doubled compared to the standard growth curve. The optimum concentration of i-PRF and Aloe Vera in combination resulted in cell growth of approximately 0.6 nm ± 0.05 nm. These findings suggest that the best concentration of Aloe Vera is 8.5 µl/ml for optimal results. However, when the concentration of Aloe Vera increased to 10 µl/ml, cell proliferation decreased despite the combination with i-PRF.

Graph 1 shows that the combination of Aloe Vera and i-PRF resulted in double the growth of HFSCs compared to the other two groups. The doubling rate of cell growth from 0.3 nm to 0.6 nm was observed with i-PRF and Aloe Vera. Therefore, the study results confirm that *Aloe Vera at an optimum concentration of 8.5 µl/ml has a synergistic effect when used with i-PRF* to achieve maximum HFSCs proliferation. The growth factors in i-PRF and the anti-inflammatory properties of Aloe Vera were found to be beneficial in an in-vitro setup.

Aloe Vera contains multivitamins, minerals, sugars, and various enzymes, but its most important properties are its ability to reduce inflammation and inhibit bacterial and fungal growth. These anti-inflammatory properties are due to the Tocotrienol component of Aloe Vera. Given these known properties of Aloe Vera, it can stimulate hair growth when used in combination with growth factors.[13] i-PRF is well known for its sustained release of growth factors, which are capable of promoting the migration and proliferation of various cell types.[14] Therefore, combining the anti-inflammatory property of Tocotrienol with the cell proliferative property of PRF can result in better HFSCs proliferation.

After evaluating all three groups, it was found that Aloe Vera at a concentration of 8.5 µl/ml exhibited the maximum cell growth both alone and in combination with i-PRF. Thus, Aloe Vera proves to be a useful adjunct to i-PRF in promoting HFSCs proliferation.

Limitation

However, it should be noted that this study was conducted in an in-vitro setup and further in-vivo clinical studies are required to validate these results.

Conclusion

The combination of i-PRF and Aloe Vera at an optimum concentration of 8.5 µl/ml has shown a synergistic effect on HFSCs proliferation, which could potentially be beneficial for hair regrowth.

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Graph

Graph I is available in the Supplementary Files section.

Figures

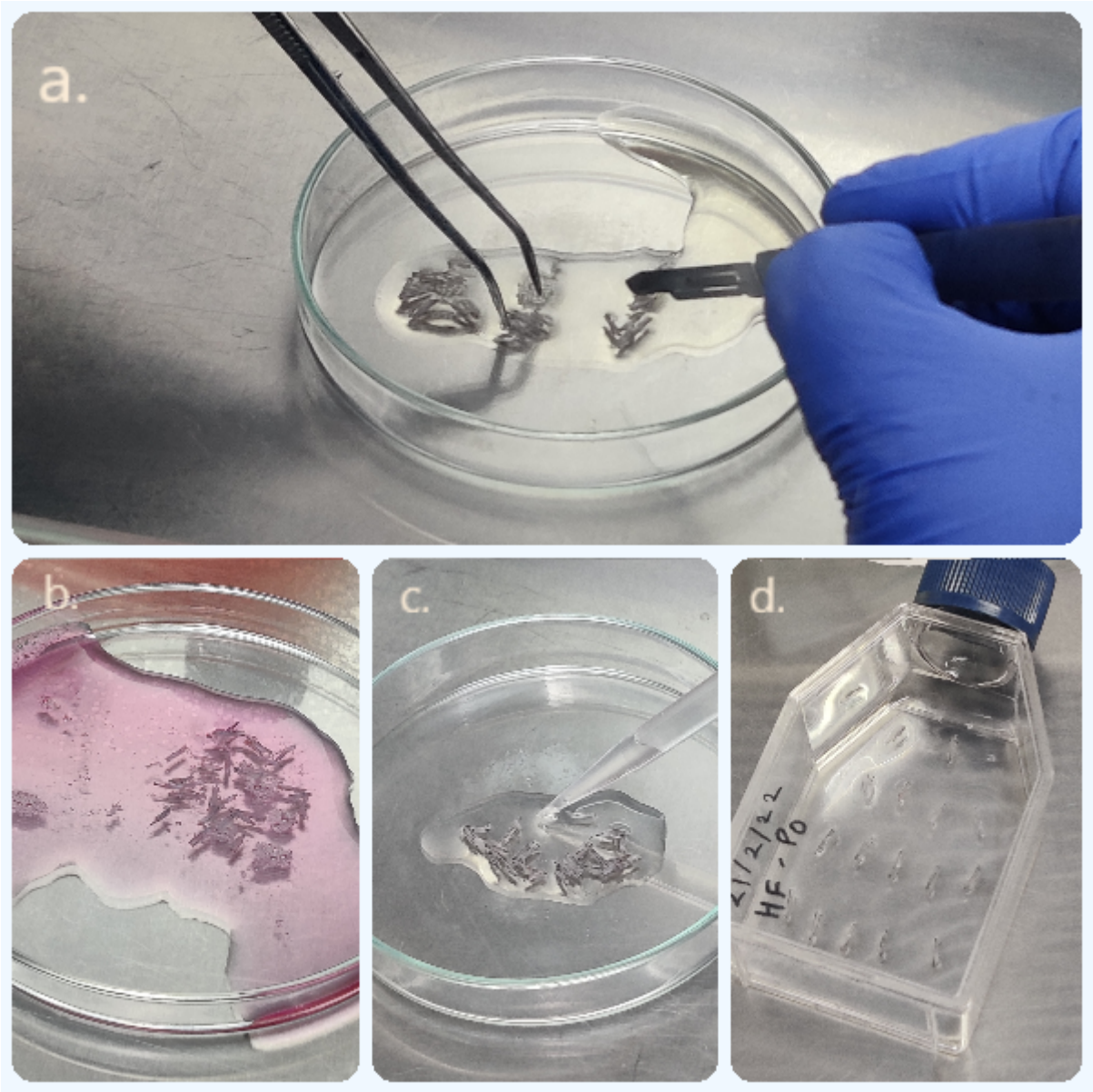

Figure 1

Different stages of hair follicle treatment in regenerative medicine laboratory.

a. Hair follicles traumatized using blade no. 15 for activation.

b. Hair follicles submerged in phosphate-buffered solution media.

c. Treatment of hair follicles is done using an anti-mycotic, anti-microbial solution.

d. Treated hair follicles are transferred to a flask submerged in DMEM.

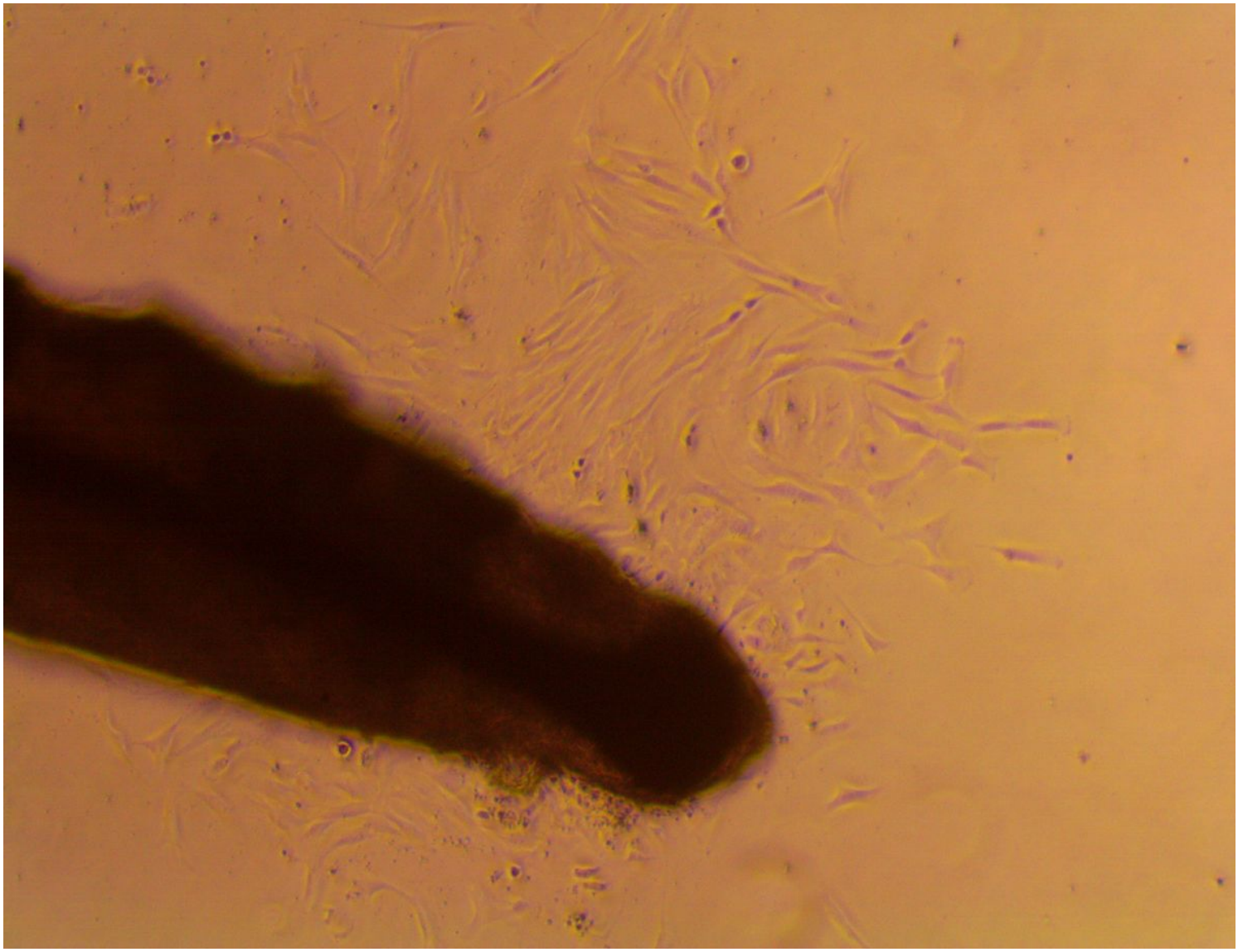

Figure 2

Stem cell isolation and characterization from a hair follicle.

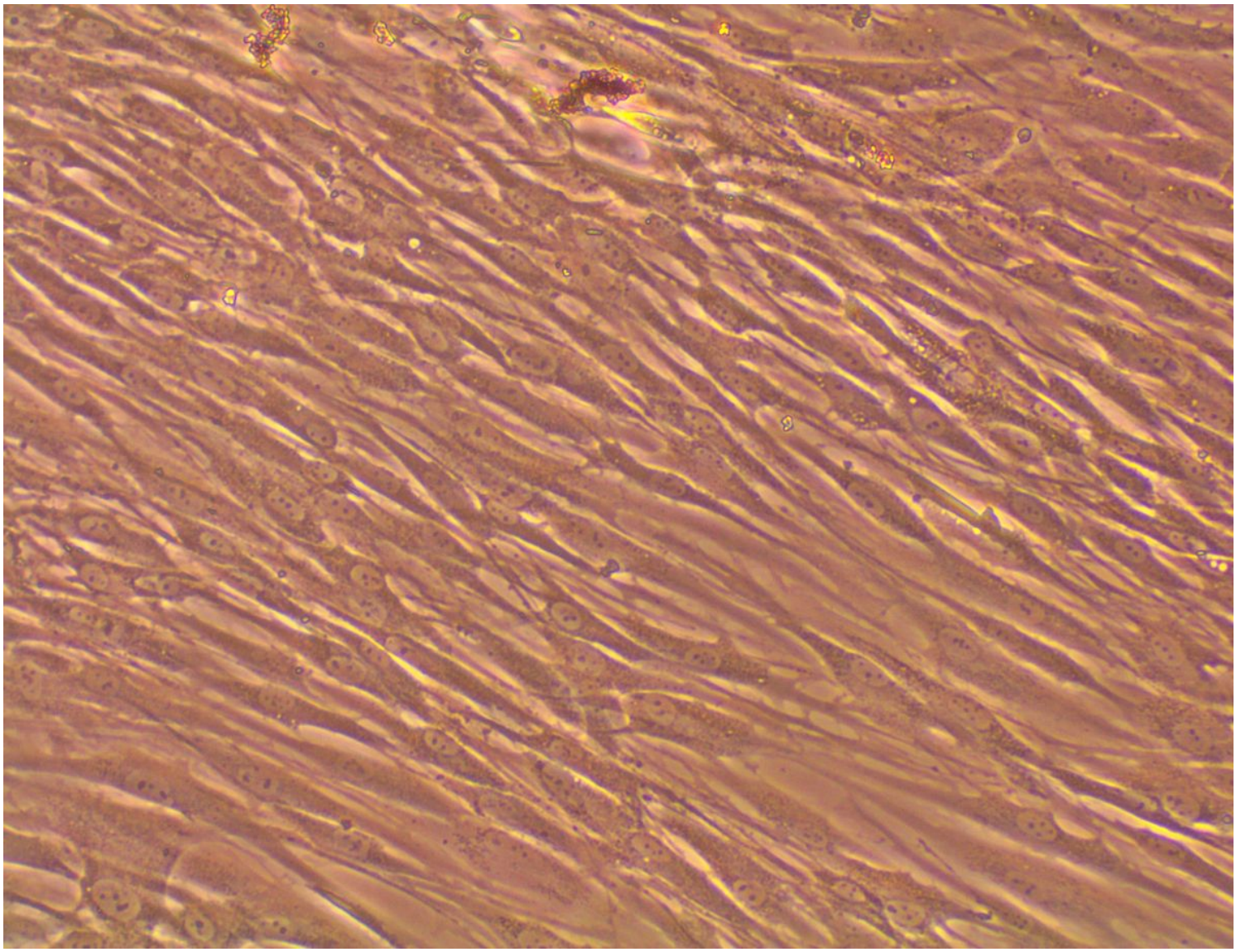

Figure 3

Proliferation of HFSCs after subjecting it to the different groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphI.png