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# Intraepidermal Injections of Autologous Epidermal Cell Suspension: A New Promising Approach to Dermatological Disorders. Preliminary Study

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## Abstracts

Regenerative medicine is a modern approach of dermatological treatment, using Epidermal Cells of the interfollicular epidermis (ESC) for their effect in skin regeneration in chronic ulcers and burns, melanoma, vitiligo, junctional epidermolysisbullosa.

Intraepidermal injections of autologous epidermal cell suspension can be a new and very promising treatment for mano other cutaneous disorders as non scarring alopecia (alopecia areata, androgenic alopecia) or scarring alopecia (Lichern Plano Pilaris alopecia, Discoid Lupus Erithematosus alopecia), anti-aging therapies. The intraepidermal injection of an autologous epidermal cell suspension is a simple, fast and safe surgical procedure: a small, thin portion of the epidermis of the patient undergoes a treatment where a suspension with all the cells collected from the epidermis is injected into the skin.

The epidermal grafts were then incubated in a trypsin solution (Trypsyn 0.5 g/ EDTA 0.2 solution, Sigma-Aldrich co) for 45 minutes at 37°C (Plasmatherm Barkley). After incubation, the trypsin solution was discarded and the tissues were washed with HBSS. The epidermis (thin yellow layer) was treated with a scalpel blade to separate the cells. The supernatant was suctioned through a sterile syringe and then cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, Saint Louis, USA) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (EuroClone, Devon, UK) and 1% penicillin/streptomycin solution (Sigma Aldrich) at 37 °C in a humidified atmosphere of 95% air and 5% $CO_2$. The cells were seeding in a 25°C at day 0, the cells showed a heterogeneous appearance with regard to shape and size and their nucleus was almost detectable.

At day 7, the cells maintained a heterogeneous appearance with regard to shape and size, even though more spheroidal elements were observed. Cells were generally in suspension, only some microspots of fibroblastoid-shaped cells were well attached to the flask surface $m^2$ cell culture flask at a density of about 20000 cells/$cm^2$.

Our preliminary study show that an suspension contains a significant number of viable cells (59,40%, SD ± 6.07%) that survive at day 7 in culture (63.55% , SD ± 5.41%).

The number of epidermal cells in each sample is significant as keratinocytes (in greater quantity) and melanocytes (1900 ±178 melanocytes/$mm^2$ as reported by Khodadadi L) (6) are detected.

Our research is currently continuing and it is focusing on the typing of the different cells in the suspension and evaluating the presence and the nature of stem cells.

**Keywords: Epidermal Cells Solution; Epidermal Stem Cells; Skin Disorder; Alopecia Areata; Intraepidermal Injection**

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## Introduction

Regenerative medicine is a modern approach of dermatological treatment, using Epidermal Cells of the inter follicular epidermis (ESC) to help skin regeneration after severe damages, such as chronic ulcers and burns [1]. Some clinical trials have already investigated the use of ESCs to treat other skin diseases such as melanomas [2-5], Vitiligo [6-10] and Junctional Epidermolysis Bullosa [11].

The intra epidermal injection of an autologous epidermal cell suspension may be a new and extremely promising treatment for several other skin disorders, such as non scarring alopecia (Alopecia Areata, Androgenic Alopecia) and scarring alopecia (Lichen Plano Pilaris Alopecia, Discoid Lupus Erithematosus Alopecia), and anti-aging therapies. Our experience in autologous preparation like PRP (platelet rich plasma) in Alopecia Areata [12] (a common auto-immune condition which causes inflammation-induced hair loss with only very limited treatment options) has encouraged us to that autologous derived material could be useful for skin regeneration. In this way started to investigate the effectiveness of epidermal cells against certain specific skin disorders. Skin is a complex structure with two different layers (the epidermis and the dermis) made up of different cell lineages which ensure the maintenance of the normal skin homeostasis (tissue repair, barrier function). In the basal layer of the epidermis, the cell population is heterogeneous as this is made up of several cell types such as keratinocytes, melanocytes, Langerhans cells, Merckell cells [13]. In the follicular epidermis multiple populations of Stem Cells (SCs) are found in different locations, with niches of SCs in the basal layer of the epidermis, around the sebaceous gland and in the bulge niche of hair follicles [14,15].

The intra epidermal injection of an autologous epidermal cell suspension is a simple, fast and safe surgical procedure: a small, thin portion of the epidermis of the patient undergoes a treatment where a suspension with all the cells collected from the epidermis is injected into the skin.

The first step, however, is to select the cells to be injected. So, the goal of this preliminary study is to investigate the content of an autologous suspension of epidermal cells and their viability also after 7 days of culture.

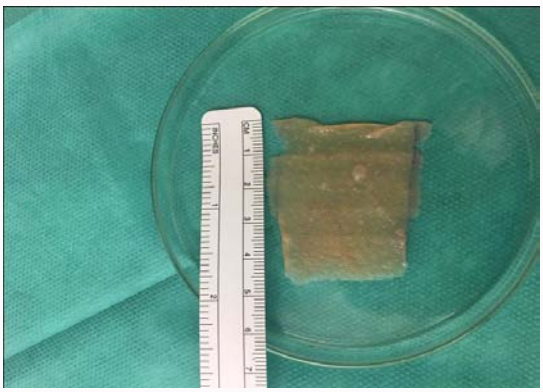

**Figure 1:** Skin graft.

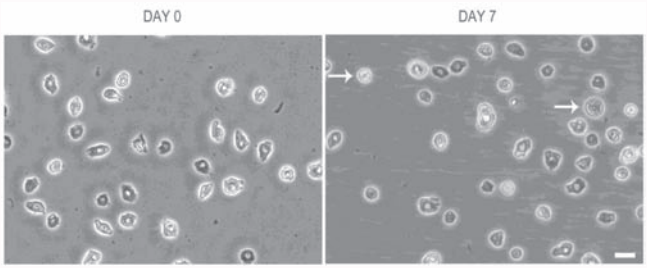


**Figure 2:** Epidermal cells at day 0 and day 7 of culture. White arrows indicate spheroidal cell elements. Bar=20 um

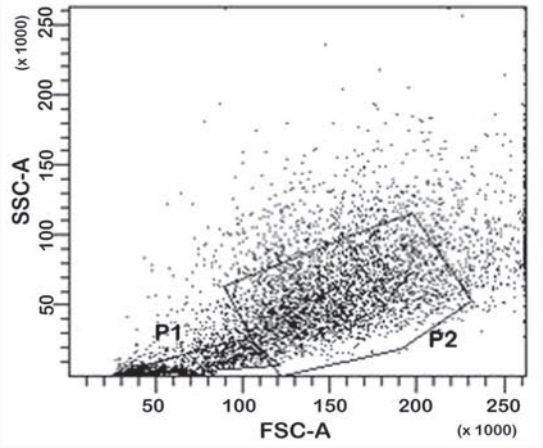


**Figure 3:** Cytofluorimetric analysis. A representative dot-plot of cell morphology at day 0. Gating cells in P1 are debris and death cells, gating cells in P2 are viable cells.

## Materials and Methods

### Collection of skin grafts

A graft of skin was collected from 10 healthy volunteers who agreed to undergo the study. All subjects provided their written informed consent before participating in the study, and the study was performed according to the Declaration of Helsinki.

About six-ten square centimeters of 0.76 mm (Figure 1) thick skin were collected from the buttock of each subject under local epidermal anesthesia (lidocaine cl 20 mg/ml, 2 ml per area) through a Zimmer Biomet Electric Dermatome. The buttock area was chosen to strongly reduce the formation of scars and causes milder cosmetic discomfort.

Each sample was immediately immersed in a 70% ethanol solution for 30 s to reduce contamination and washed with HBSS twice. The epidermal grafts were then incubated in a trypsin solution (Trypsyn 0.5 g/ EDTA 0.2 solution, Sigma-Aldrich co)for 45 minutes at 37 °C (Plasmatherm Barkley). After incubation, the trypsin solution was discarded and the tissues were washed with HBSS. The epidermis (thin yellow layer) was treated with a scalpel blade to separate the cells. The supernatant was suctioned through a sterile syringe and then cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, Saint Louis, USA) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (EuroClone, Devon, UK) and 1% penicillin/streptomycin solution (Sigma Aldrich) at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$. The cells were seeding in a 25 $cm^2$ cell culture flask at a density of about 20000cells/$cm^2$.

Cell morphology was evaluated before the seeding and after a 7 day culture by using an inverted Olympus microscope.

### Cytofluorimetric analysis for cell viability

Cells were centrifuged at 1000 rpm for 5 minutes. The supernatant was then removed. The pellet was re-suspended in 3 ml 4% para formaldehyde in phosphate buffer 0.1 M pH 7.4 for fixation. After 20

minutes at +4°C, the cells were centrifuged at 1000 rpm for 5 minutes and the pellet was re-suspended in 500 µl PBS. Finally, the cell samples were analysed with FACS (BD FACSCanto™ BD Bioscience, San Jose, CA) and the data were analysed by the BD FACSDiva™ software version 8.8.7 (BD Bioscience, San Jose, CA).

An analysis was performed at day 0 and day 7 after the culture. The viable cells were identified using morphological parameters by choosing a range of cell size combined with morphological cell complexity.

## Results

### Cell morphology

The morphology of the cells was observed through an inverted microscope (Figure 2 and 3).

At day 0, the cells showed a heterogeneous appearance with regard to shape and size and their nucleus was almost detectable.

At day 7, the cells maintained a heterogeneous appearance with regard to shape and size, even though more spheroidal elements were observed. Cells were generally in suspension, only some microspots of fibro blastoid-shaped cells were well attached to the flask surface.

### Cytofluorimetric analysis for cell viability

The analysis at day 0 showed that the percentage of viable cells was 59.40% (SD ± 6.07%), while the percentage of non-viable cells was 16.48% (SD ± 5.18%). The remaining cells formed clusters.

At day 7, the percentage of viable cells was 63.55% (SD ± 5.41%), while the percentage of non-viable cells was 15.50% (SD ± 5.68%). The remaining cells formed clusters.

These results show how the absolute percentage of viable and non-viable cells is maintained after a 7 day culture.

The numbers of isolated cells at day 0 are shown in (Table 1).

**Table 1:** Numbers of isolated cells at day 0.

| Subject | Donor surface area ($cm^2$) | # epidermal cells X $10^3$ |
|---|---|---|
| 1 | 5.9 | 325 |
| 2 | 6.3 | 475 |
| 3 | 5.7 | 720 |
| 4 | 6.2 | 483 |
| 5 | 4.6 | 700 |
| 6 | 5.3 | 580 |
| 7 | 6.1 | 650 |
| 8 | 5.7 | 734 |
| 9 | 6.2 | 580 |
| 10 | 4.7 | 315 |
| average | 5.7 | 556.63 |

## Discussion

Autologous epidermal cell suspension injected into the skin may well be a new approach to treat several skin disorders.

Regenerative medicine could be applied in several medical fields, and dermatology may use this therapy to successfully treat specific disorders.

This preliminary study shows that an autologous epidermal cells suspension contains a significant number of viable cells which survive at day 7 in culture.

The number of epidermal cells in each sample is significant as keratinocytes (in greater quantity) and melanocytes (1900 ±178 melanocytes/$mm^2$ as reported by Khodadadi L) [6] are detected.

Our research is currently continuing and it is focusing on the typing of the different cells in the suspension and evaluating the presence and the nature of stem cells.

If biological studies also confirm these data, it is reasonable to think that the surgical procedure with an autologous epidermal cell suspension may offer several interesting opportunities for the near future, as:

1) It is simple and fast (the full procedure lasts 60 – 90 minutes)

2) The risk of side effects is very limited (scars in the donor area, pain in the treated area due to the injections)

3) It is safe (no risk of oncogenic disorders, no immunologic reaction)

4) It is not expensive

5) It is an outpatient treatment.

However, some further studies are still needed to validate our findings in a larger cohort of skin samples and focus on the typing of the cells in the suspension. For the same reason, the actual effectiveness of this procedure needs to be validated by some controlled and randomized clinical trials.

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