Brief Communication

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Stem Cell Niche is Partially Lost during Follicular Plucking: A Preliminary Pilot Study

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ABSTRACT

Background: Clinical hair transplant studies have revealed that follicular unit extraction (FUE) is superior in terms of stable hair growth in comparison to follicular plucking (FP). Various reasons have been cited for this clinical outcome. FUE and FP are employed to obtain the hair follicle units for hair transplant and recently for cell based therapies in vitiligo. However, there is no scientific data available on the comparison of stem cell fraction in the cell suspension obtained by FUE and FP. Therefore, we undertook this study to compare the percentage of stem cells in the hair follicle obtained by FUE and FP. **Objective:** The purpose of the following study is to evaluate the quantitative stem cell pool in the hair follicle obtained by FUE and FP. **Materials and Methods:** A total of 3 human subjects were enrolled with age groups of 17-25 years. Both methods of tissue harvest: FUE and FP; were employed on each subject. There was no vitiligo lesion on the scalp in any of the patients. Hair follicles were incubated with trypsin-EDTA solution at 37°C for 90 min to separate outer root sheath cells. The cell suspension was passed through a 70 μm cell strainer; filtrate was centrifuged to obtain the cell pellet. Cells were labeled with cluster of differentiation (CD200) antibody and acquired with flowcytometry. **Results:** The mean percentage of CD200 positive cells in FUE and FP method come out to be 8.43 and 1.63 respectively (P = 0.0152). **Conclusion:** FUE is a better method of the hair follicle harvesting for cell based applications as the stem cell fraction is significantly higher in comparison to FP.

Key words: Cluster of differentiation, follicular plucking, follicular unit extraction, stem cells

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INTRODUCTION

Many adult tissues harbor stem cells. Cotsarelis *et al.* highlighted the bulge as a niche for hair follicle stem cells; which has led to many developments in the area of follicular stem cell biology.[1]

Hair follicle is an appendage organ of the skin which is of importance to the survival of mammals and maintains significance for the human race – not just biologically, but also cosmetically and commercially.[2] The hair follicle offers an interesting and valuable model for studying stem cells as it undergoes cyclic changes and is least invasive source of stem cells.[1,3-6]

Hair follicle derived outer root sheath (ORS) cell suspension has translational value. Vanscheidt and Hunziker have demonstrated effective treatment of vitiligo using single cell suspension of plucked hair follicle.[7] Non cultured extracted hair follicle ORS cell suspension has been used for the first time resulting in significant repigmentation in vitiligo patients, by our group[8] Ohyama *et al.* have shown that cluster of differentiation ($CD200^+$) cells obtained from the hair follicle show high colony-forming efficiency in clonogenic assays, which indicates that CD200 can be a potential surface marker to identify and purify the human hair follicle bulge stem cells.[9]

The increased interest in the use of cell based therapy in clinical settings, such as transplantation of melanocytes in patients with pigmentary disorders, cultured skin substitutes etc., has necessitated the process of getting the optimum yield of cells.[10]

The primary aim of the present study was to evaluate method of obtaining hair follicle samples which contains adequate number of stem cells and does not leave behind a significant scar at the donor site.

MATERIALS AND METHODS

Tissue samples

Hair follicles were obtained from the subjects after taking their written informed consent as per the Institutional Ethics Committee and Institutional Committee for Stem Cell Research and Therapy Guidelines of All India Institute of Medical Sciences (AIIMS), New Delhi, India.

Patients coming to the Outpatient Department (OPD) of Dermatology, AIIMS were enrolled as subjects, after taking their educated consent, to obtain the hair follicle by plucking and follicular unit extraction **(**FUE). The follicles obtained by FUE were used for the surgical treatment of stable vitiligo, as well.

Hair follicle harvest

Both procedures were carried out on an out-patient basis under local anesthesia from patients attending to OPD of Dermatology, AIIMS. Samples were obtained for processing in transport media within 15-20 min of harvesting of the tissue. Both methods of tissue harvest; FUE and follicular plucking (FP); were employed in each of the subjects. There were no vitiligo lesions on the scalp in any of the patients. Samples were taken from occipital area of the scalp.

FUE

The FUE was done as described by Rassman *et al*.[11] In brief, hairs were trimmed to a length of approximately 2 mm. Nearly 2% lignocaine was infiltrated in the area for local anesthesia. To obtain follicular units, 1 mm punch was rotated till mid-dermis in the direction of the hair follicle care was taken not to go up to subcutaneous space to avoid transaction of the hair follicle. Then follicular unit was pulled out using hair follicle holding forceps by holding skin surrounding hair sample. Approximately 15-20 pigmented follicles were extracted per subject and collected in transport media, containing Dulbecco's modified eagle's medium (Sigma, St. Louis, MO, U.S.A.) pH = 7.2 supplemented with penicillin, streptomycin and amphotericin-B (Gibco BRL, Gaithersburg, MD, U.S.A.). The procedure took approximately 30-45 min/case.

FP

For hair plucking a rubber tipped depilation forcep was used. In this technique, hair were held firmly and pulled briskly. Approximately 60 pigmented follicles were plucked per patient and collected in transport media. The procedure took approximately 5-10 min/case.

Preparation of single cell suspension

The extracted or plucked follicles were transported to the laboratory under sterile conditions and washed three times with phosphate buffered saline containing the antibiotics and antimycotics (Gibco BRL, Gaithersburg, MD, U.S.A.). Single cell suspension from the follicles were prepared with 0.25% trypsin-0.05% EDTA (Gibco BRL, Gaithersburg, MD, U.S.A.) at 37°C for 90 min to prepare the single cell suspension. The cell suspensions of all the three tubes were filtered through a 70 μm cell strainer (Becton Dickinson, Sunnyvale, CA, U.S.A.) to prepare single cell suspension. Finally, the cell suspension was centrifuged for 5 min at 1000 rpm to obtain a concentrated hair follicle suspension.

Flowcytometry of the cell suspension

Cells were labeled with PE mouse anti-human CD 200 antibody (BD Biosciences-Pharmingen). Marker for the negative population was set with the use of PE mouse IgG1κ antibody (BD Biosciences-Pharmingen) and unstained cell population. After labeling, cells were acquired on a Becton Dickinson LSR II flowcytometer with the use of 488-nm argon ion laser and analyzed by biosciences-pharmingen (BD) FACS Diva software version 6.1.2. For each sample, at least duplicate acquisitions were performed.

Viability and cell count of cell suspension

The viability and cell count of the cells suspension obtained after trypsinization by both the method was checked by trypan blue dye exclusion method inside neubauer chamber**.**

RESULTS

Cells extracted from the follicles obtained by both the methods were analyzed for the presence of stem cell marker CD200 by the flowcytometry [Table 1 and Figure 1]. The mean of CD200 positive cells in FUE and FP method was obtained to be 8.43 and 1.63 respectively ($P = 0.0152$).

The average cell yield per follicle for the FUE and FP method was found as 2500 and 250 respectively. Average viability of the cells obtained was 92% for both methods.

DISCUSSION

There are various methods of choice to harvest the hair follicles for hair transplant or for cell based therapies. If we compare the scalp biopsy and FP the former may lead to a visible scar on the scalp and is associated with greater

Table 1: Comparison of cluster of differentiation 200⁺ cells in outer root sheath cell suspension of hair follicles obtained by follicular unit extraction and follicular plucking method

Gender	Age (years)	FUE method %CD200⁺ cells	Follicular plucking method %CD200⁺ cells
Male	25	11.5	1.5
Male	20	7.8	2.4
Female	17	6	1

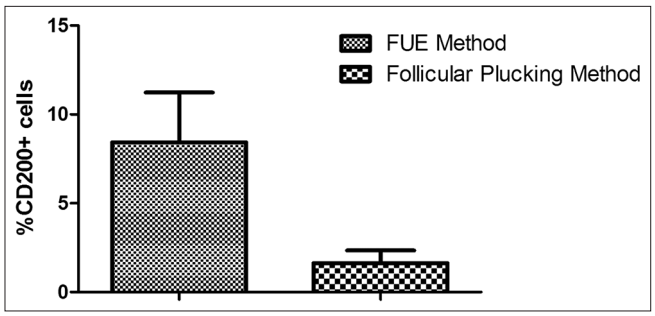

Figure 1: Enumeration of stem cells (cluster of differentiation [CD200⁺]) in hair follicle harvested by follicular unit extraction (FUE) and follicular plucking (FP) method. Mean ± SD of 4 samples for CD200⁺ cell yield by FUE and FP method

tissue loss whereas the latter may be associated with the loss of the stem cell, mostly located in the bulge area of the hair follicle.[7,12] The procedure of FUE involves removal of much less volume of tissue in comparison to scalp biopsy and hence, results in relatively invisible scarring and less healing time.[13] Though, the plucking of the hair follicle is faster, non-invasive and less painful to the patient; the cell yield is low for which we need more number of follicular units. However, same number of cells can be obtained with few follicles obtained by FUE as is evident in our study.

In a study by Gho *et al*. have reported expression of stem cell markers in *in vitro* cultured plucked hair follicle.[14] However, clinical studies by the same scientific group have shown that plucked hair transplantation does not result in normal hair growth at the recipient site in contrast to FUE grafts.[14,15] One of the reasons might be the retention of the majority of follicular stem cells, which are responsible for hair growth, in the skin, in case of plucked hair, whereas they are present in extracted hair follicles.

Ohyama *et al*., have reported the presence of 15-20% CD 200 positive cells in mid human hair follicle ORS cells by flowcytometry (sample size = 05 human scalp biopsy).[9] The stem cell pool also varies depending upon the stage of the angan follicle as shown by Zhang *et al*. They have shown that self-renewal of bulge cells in late anagen replenishes the niche after depletion due to the recruitment of cells in early anagen, to form the hair follicle.[16]

These, observations show that the number of CD200 positive cells in the ORS varies and it also depends upon the stage of the hair follicle.

In our observation, intraspecific variation is large which may be due to stage of the anagen hair follicle plucked. The sample size studied, in the present study, is again very small and larger study data is needed.

The results obtained from the above preliminary pilot study may pave the way for a newer treatment modality using transplantation of ORS cells from extracted hair follicles. Use of this minimally invasive technique with no visible residual scarring might be more acceptable to the cosmetically enlightened population when compared with other sites of tissue harvest such as blister, punch biopsy and shave biopsy from skin to be used for cell based therapy. More studies with clinical application ORS cell suspension of the HF obtained by FUE and further expansion and differentiation of cells in the desired cell types are needed.

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