Treatment of Androgenetic Alopecia with Autologous CD200 Positive Cell Suspension

Dear Editor,

Androgenetic alopecia (AGA) is a chronic disorder associated with miniaturization of hair. Research has shown that in AGA, the number hair follicular stem cell number remains the same but the number of proliferating progenitor CD200 and CD34 positive cells is reduced.[1] Gentile *et al.*[2,3] have performed CD200-rich progenitor cell transplant by autologous micrografts using manual mechanical detachment and mechanical device Rigenera™.

Non-cultured melanocyte transplantation (NCMT) is a well-established procedure for treatment of vitiligo. Trypsin enzyme is used to prepare a cell suspension of melanocytes and which is applied to dermabraded vitiliginous skin. Gupta *et al.*[4] have used hair follicles as a source of melanocytes. They used trypsin to separate the melanocytes from hair follicles. They have shown promising results in vitiligo. Incidentally, they found the presence of CD200 positive progenitor cell in their suspension indicating that same suspension may be useful in treatment of AGA. This is a retrospective report of a case where trypsin-isolated single cell suspension was used to treat AGA. A flow cytometric analysis was done.

One patient, aged 22 years, having grade III androgenetic alopecia, was treated with cell suspension of occipital follicular units. 24 mL blood was drawn to prepare platelet-rich plasma (PRP). The patient underwent follicular unit extraction (FUE) of 25 hair follicles (HF) using 0.9 mm punch. HF were washed with saline and collected in DMEM (Dulbecco's modified Eagle's medium) (Melanotrans kit, Cryobank Fertility Research Center, Jalna, Maharashtra, India). HF were incubated in 0.25% trypsin-EDTA (Melanotrans kit) at 37°C for 60 min. Before adding HF to trypsin, they were split longitudinally, slicing through the outer root sheath, to expose the bulge area to the trypsin. During incubation the HF were shaken every 5 min achieving an efficient separation of cells. After 1 h, only keratinous shafts remained in the suspension [Figure 1] and trypsin was inactivated using trypsin inhibitor. The suspension was filtered through a 40 µ filter ensure a single cell suspension. The suspension was then pelleted and redissolved in 5 mL of PRP. 0.05 mL was injected per cm^2 and hair growth was measured using trichoscopy before the procedure and after 8 weeks. Cell suspension from one case was sent for flow cytometry to ascertain the number of CD34 positive cells and CD200 positive cells. HF cells were washed with phosphate buffered saline (PBS) and suspended in 100 µL staining buffer (0.5% bovine serum albumin in PBS). 1 µL of antibody (FITC anti-human CD34 and PE anti-human CD200, BioLegend, San Diego, California) was added per

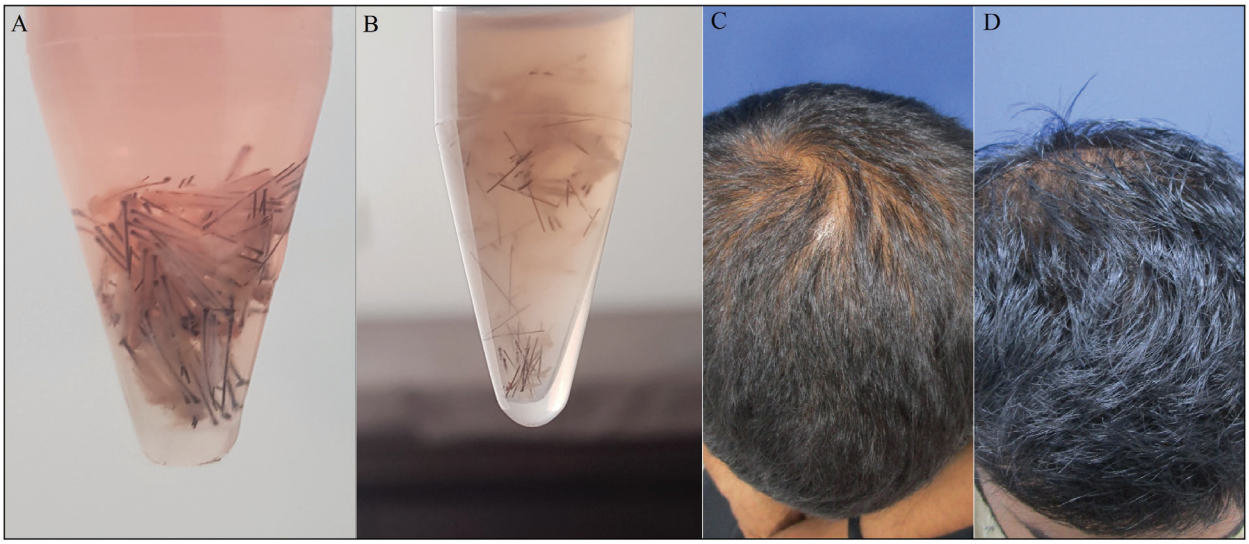

Figure 1: (A) FUE hair follicles before the start of trypsin cell separation. (B) FUE hair follicles after complete disintegration by trypsin. (C) Photograph of the vertex of patient with androgenetic alopecia before treatment. (D) Photograph of the vertex of the patient with androgenetic alopecia 8 weeks after treatment

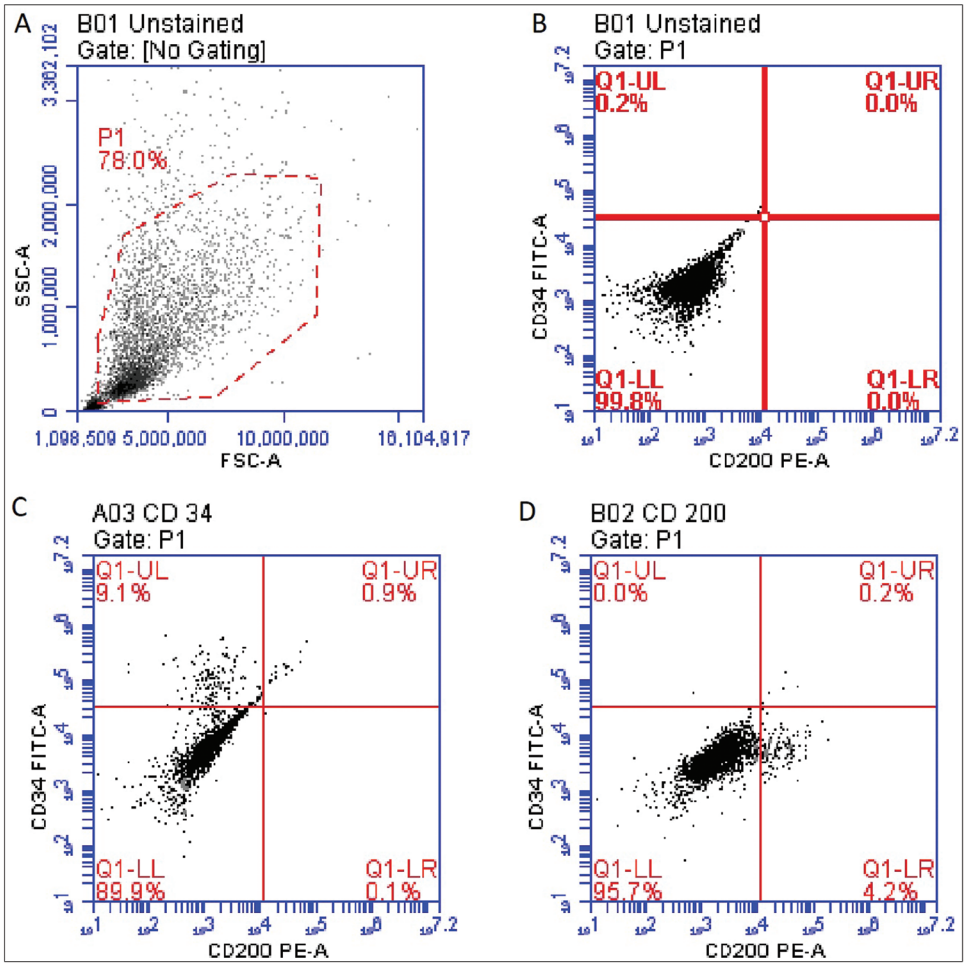

Figure 2: Flowcytometry was done using BD Accuri C6 plus flow cytometer (Becton, Dickinson and Company, Franklin Lakes, New Jersey). (A) The gating strategy developed with the help of unstained cells to identify overall hair follicle cell population and to remove cell debris from analysis. (B) Unstained cells were used to set quadrant gates for quantification of CD34 +ve and CD200 +ve cells. Compensation for fluorescence spill over was carried out using single stained cells. (C) Among the hair follicle cells 9.1% cells were CD34 positive 2D. 4.2% cells were CD200 positive

1×10^5 cells and the cells were incubated in dark for 15 min at room temperature. CD34 +ve and CD200 +ve cells were quantified using flow cytometer. Result showed an increase in hair density of 21 hairs/cm^2. On flowcytometry, 9.1% cells were CD34 positive and 4.2% cells were CD200 positive in the suspension [Figure 2].

In this case, we have quantified CD34 positive and CD200 positive hair follicle progenitor cells. The mechanical separation of cells using Rigenera™ and mechanical detachment of cells as shown by Gentile *et al*., utilize skin biopsy samples from the scalp as a source of progenitor cells and have shown 2.6±0.3% CD200 positive cells in their suspension. A possible higher yield in our case (4.2%) was probably because of use of FUE hair follicles instead of using skin biopsy samples. The innovative method of splitting open the outer root sheath to expose cells in the bulge area also possibly allowed a higher yield. We have repurposed the traditional method of NCMT for AGA. Further studies are required to confirm the viability of the use of this method in the treatment of AGA.

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Conflicts of interest

There are no conflicts of interest.

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