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Bone Marrow Mesenchymal Cells (BMSC) With PLGA Promotes Hair Growth in Wistar Rats

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I. Introduction

Hair is one of the important derivatives of skin and has high cosmetic value among present human population. Hair in each part of the body have definite period of growth, after which they shed and replaced. Shedding of 50-100 hairs a day on average is considered to be normal, which will be followed by normal hair growth. (**1**)Hair growth is a cyclical process which occurs with phase of anagen, catagen and telogen. Each phase of hair cycle is independent with anagen - growth phase, lasting for 3 – 5 years. Catagen – short transitional phase, of 10 days, where the hair follicle detaches itself from the nourishing blood supply. Telogen – shedding phase, where the hair follicle is released following inactive 30 days of hair cycle. (**2**) Hair is not only aesthetic but its loss can create depression and anxiety.(**3**)Hair loss happens when internal milieu of hair growth cycle is lost.Alopecia is due to factors like radiation, stress, hormonal, hereditary, improper nutrition, infection, hair style and treatment. These factors disturb the hair growth cycle by decreasing stem cell activity in hair bulb and hair follicle regeneration capacity.(**4**)Development of hair follicle arises from dermal papillary cells which intern formed from dermal condensate (**5**) hair bulb containing dermal papillary cells, which induct and regulate the formation of new hair follicles.(**6**) The cells present in the hair bulb grows to form hair shaft. Hair shaft contains outer cortex and inner medulla. Cortex of hair containing keratinised cells with melanin, while vacuolated cell in the medulla. Induction of hair follicle is initiated by bone morphogenic protein.(**7**)Hair bulb contain basal layer of epithelial cell also accommodate stem cell. This cells proliferate to reach and make inner root sheath and hair shaft. Hair follicular stem cells in the basal layer take part in regeneration of epidermal cell, hair follicle and sebaceous gland.(**8**).The stem cell buried in the basal layer is invaginated by papillary connective tissue with fibroblast which provide nutrition to the growing cells. Erector pili muscle is attached to portion of hair follicle called bulge. The bulge contain epithelial and melanocyte stem cells. Bulge region is divided into upper and lower part. The lower part of the bulge contain progenitor cells which migrate down to the hair matrix and form hair stem and internal hair follicle. (**9**)Stem cell plasticity has created great interest because of its potential therapeutic application in regenerative medicine. BMSCs are supposed to be the ideal transplantable cell, due to its easy accessibility, rapid expansion capacity when cultured and immuno-privileged nature. (**10**)Animal models prepared by removing a patch of hair and transplanted with bone marrow mesenchymal stem cells and PLGA would potentially improve hair growth by treating the cause.

II. Subjects and Methods

Selection of Experimental Animals:

Randomly breed male Wistar albino rat (150-200gms) were used for the study. 18 Rats were kept in polypropylene cages (3 per cage) with sterilized and dry paddy husk as bedding material. The animals were fed with commercial laboratory animal feed (TANUVAS-Chennai) and purified water *ad libitum.* The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA, India). This study has approval of institutional animal ethical committee.

Isolation and Culture of BMSc's

Six weeks old Wistar weighing 80gms was used for the isolation of BMSCs. (**11**) The Wistar rat was sacrificed using diethyl ether and bones were collected under sterile conditions; muscles, connective tissues attached to the bone were cleaned and rinsed in phosphate buffer solution. Marrow from each bone was collected by cutting the epiphyseal ends and flushing it with Dulbaco minimum essential medium (sigma - USA).After filtering , cells were centrifuged at 1000 rpm for 5min. Purified cells were finally dispersed in Dulbaco minimum essential medium with 15% fetal bovine serum (sigma - USA).

Cell culture and expansion

Isolated BMSCs were plated in T-25 cm^2 and T-75 cm^2 tissue culture flasks containing approximated stem cell nutrient medium (sigma - USA) at a density of 10 x 10^5 cells per flask. Flasks were maintained in incubator at 37^{o}C and 5% carbon dioxide. Medium was replaced every third day. Cell viability was confirmed by continuous cell division and cells were sub cultured using 3ml trypsin / EDTA (sigma- USA), when the flask reached 90% confluence.(**12**)

Flow Cytometry analysis

After initial plating, primary culture were harvested by trypsinization, and cells were fixed in neutralized 2% paraformaldehyde solution (sigma - USA) for 30min. Fixed cells were washed twice with phosphate buffer saline (PBS) (sigma -USA) and incubated with following antibodies CD90 for positive expression and CD34 for negative expression.

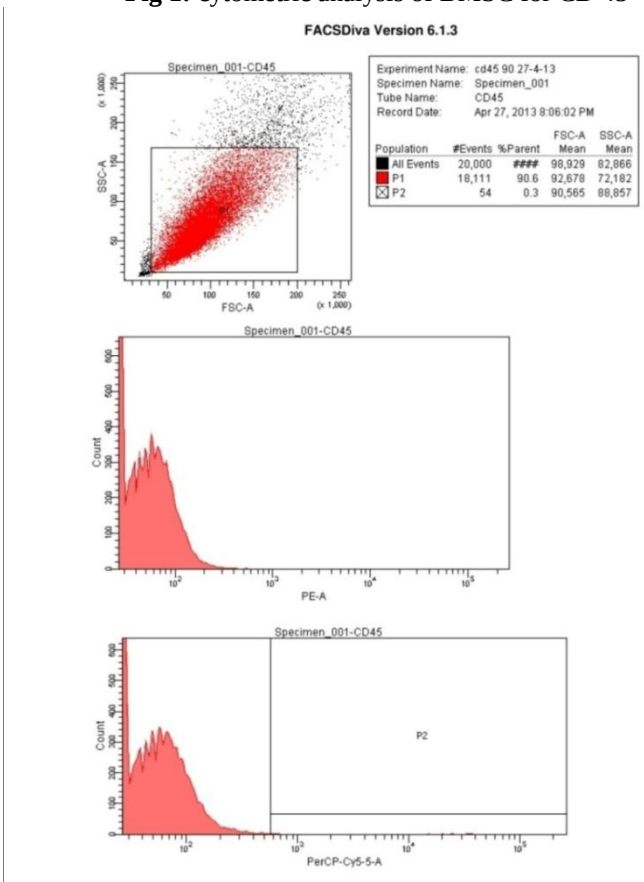
Experimental procedure

The animals were divided into three groups, 6 each. The animals were anaesthetized with ketamine (80mg/kg) and xylozine (10mg/kg) cocktail, intraperitonially. Anaesthetized animal was placed on a surgical table .Under aseptic precautions, area of size 2.5 x 2.5cm depilated with razer and marked using skin marker on dorsal aspect of the body wall. BMSCs with PLGA were loaded in microliter syringe at a concentration of 1x10^5 cells/µl. Group I - Depilated Control injected with saline. Group II - Depilated injected with PLGA. Group III - Depilated transplanted with BMSC and PLGA.

III. Results

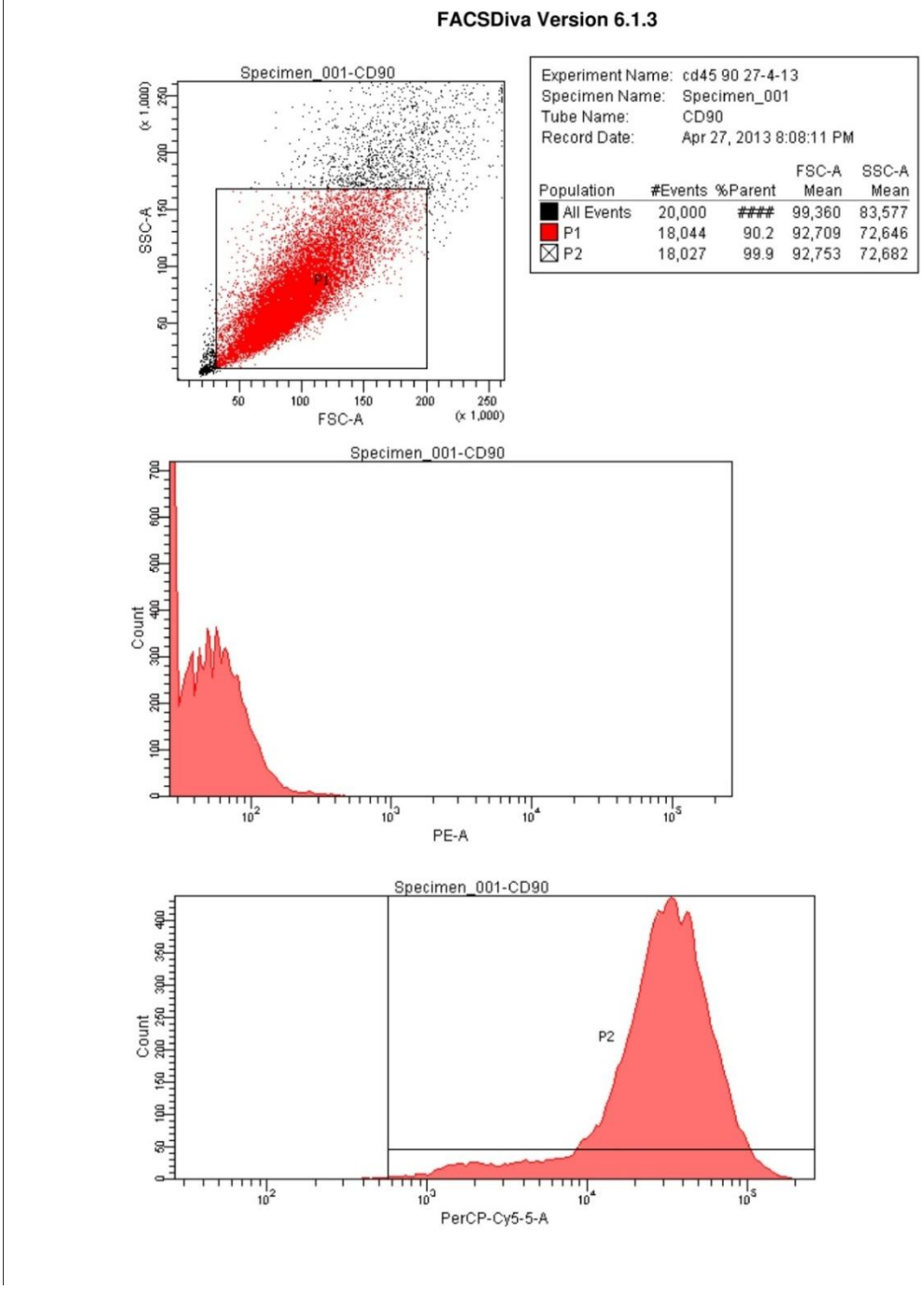
Cytometric analysis revealed CD 90 positivity and CD 45 negativity for BMSC's. (Fig: 1, 2**)**

Fig 1: cytometric analysis of BMSC for CD 45



Legend 1: Flow cytometric results of BMSC's for surface antigen of CD 45, showing negative expression.

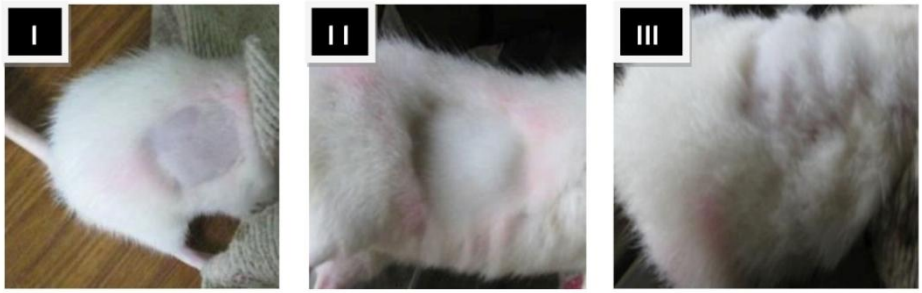
Fig 2: Cytometric analysis of BMSC for CD 90



Legend 2**:** flow cytometric results of BMSC's for surface antigen of CD 90, showing positive expression.

The efficiency of BMSC and PLGA was evaluated between group to study the hair shaft length, pattern and density. White bullae like eruption observed at 1st week transplanted with BMSC and PLGA. Such observation was absent in group treated with saline and PLGA. The hair density was very thin and the growth pattern was not clearly visible in group treated with saline at the end of 3rd Week. In group treated with PLGA the hair density was medium and growth pattern was wavy. In group treated with BMSC and PLGA the hair density was thick and growth pattern was bushy, when compared with surrounding body hair at the sixth week. (Fig: 3)

Fig 3: Observation of hair growth at the end of 6th week in group I, II &III.



Legend 3: Bushy pattern seen in group treated with BMSC and PLGA

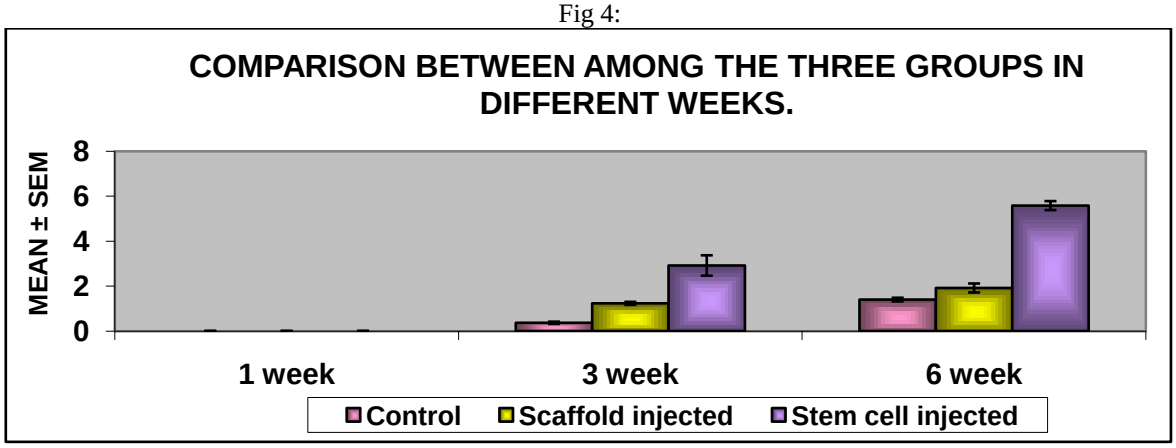
Table 1: Observation at sixth week.

OBSERVATIONS	Group I	Group II	Group III
HAIR DENSITY	Thin film of hair	Medium with sparse distribution.	Thick
GROWTH PATTERN	Not appreciated	wavy	Bushy

legend 4 :Thick hair density and busy pattern observed in animals treated with BMSC & PLGA.

Hair shaft length was measures in all the three groups at the end of 3rdweek (group I - 0.3 mm, group II-1.2mm and group III -2.9mm) and 6th week (group I – 1.4 mm, group II-1.9mm and group III -5.5mm) respectively and the results were studied using ANOVA, Post Hoc and Kruskal-Wallis Test.

Fig 4:



legend5: Average hair shaft length of each group. Each bar represents ± SEM of experimental animals. P <0.001, in group treated with BMSC and PLGA.

IV. Discussion:

Hair in each part of the body have definite period of growth, after which they shed and replaced.Hair loss happens when internal milieu of hair growth cycle is lost.The common reason for hair loss in both the sexes could be due to ageing. Ageing may be related to loss of hair follicle, due to depletion of "A" type laminin formed from LMNA gene. Type "A" laminin may carry receptors for maintenance and adaptive response to stress.(**13), (14), (15), (16**) Hair growth is a cyclical process which occurs with phase of anagen, catagen and telogen. Each phase of hair cycle is independent with anagen - growth phase, lasting for 3 – 5 years. Catagen – short transitional phase, of 10 days, where the hair follicle detaches itself from the nourishing blood supply. Telogen – shedding phase, where the hair follicle is released following inactive 30 days of hair cycle. (**2**)The present study thus uses senile animals to depict the human hair loss. Hair follicle of Wistar rats underwent phases, of Anagen, Catagen and Telogen. The rat hair follicles are more in Telogen phase, when compared to anagen phase and they show wavy pattern growth. Growth of hair follicle is influenced by various growth factors. Cytokinine expression in the hair follicle was identified in-vivo in rats.(**17)**Different growth factor families including the epidermal growth factor (EGF)-related ligands, fibroblast growth factors (FGF), transforming growth factor-beta (TGF-beta), and platelet-derived growth factor (PDGF) have been shown to be crucial for the regulation of the hair cycle and hair growth.(**18)**These factors disturb the hair growth cycle by decreasing stem cell activity in hair bulb and hair follicle regeneration capacity. (**4**)

Changes at first week

Observation on the second postoperative day shows, white bullae at the injection site for the animals belonging to group III, which was not observed in other groups. This may be due to the interaction of the mesenchymal stem cells or migration of the cells to the surrounding niche. This could also be due to active migration and signaling among the stem cell population(**19).** For cell regulation and maintenance, signaling operate in four main pathways (ie.) NOTCH, Wnt, TGF and Sonic Hedgehog. (**20)**Most notable of these are Notch and Wntsignalling, which are implicated in aging. Hair follicle loss in ageing is due to the aberrant NOTCH signalling of adult hair follicle stem cells.(**21)**

Changes at third week

There was thick hair growth in group treated with BMSC and PLGA. The length of hair shaft was long, thick and bushy, than the other groups, which differs in growing pattern. Average hair shaft length in group I - 0.3 mm, group II-1.2 mm and group III -2.9 mm respectively. Induction of hair follicle is initiated by bone morphogenic protein. (**7**)The enhanced growth in group III would have been due to interaction of stem cells with various growth factors like EGF, FGF, TGF-beta, and PDGF. Animals received PLGA revealed hair with sparse distribution and wavy pattern. Wavy pattern signifiestelogenic phase of hair growth. (**22), (23), (24), (25)**this proves that PLGA was merely a vehicle to hold BMSC and improve angiogenesis, due to its porous nature. (**13), (14), (15), (16)**in group treated with saline shows thin film of hair, sparsely distributed around depilated site. This could be due to delay in follicular cells to adopt signaling.

Changes at sixth week

Group received BMSC and PLGA showed dense, bushy and increased hair shaft in depilated area. The hair shaft length in group I – 1.4 mm, group II-1.9mm and group III -5.5mm respectively and results were studied using ANOVA, Post Hoc and Kruskal-Wallis Test.($P < 0.001$). Increased shaft length and density is due to follicle-follicle interaction, growth factors and receptors in hair follicle. Cell to cell NOTCH signaling is required for the maintenance of stem cell population. (**26**) Group treated with normal saline and PLGA showed sparse hair distribution and slow wavy patter hair growth. This must have been probably due to defective or failure in cell signaling (this area need to be further explored) .In group II even with PLGA, hair growth is not improved as in group III, this could be due to increased vascularity and defective cell signaling. Group III has fulfilled hair growth by PLGA angiogenesis and BMSC signaling. Molecular studies show that "A"type laminin present within the nucleus of adult hair follicle derived from mesenchymal stem cells gets depleted in ageing. This can be rejuvenated by injecting mesenchymal stem cells from bone marrow. This stem cell injected will invaginated papillary connective tissue with fibroblast which provide nutrition to the growing cells.Since both types of cells carry same type "A" laminin. BMSC transplantation could be ideal to rejuvenate adult hair follicle. This experimental study vividly reveals that bone marrow derived mesenchymal stem cells fasten hair growth and it's a successive tool to treat alopecia.

V. Conclusion

In this preliminary study it is observed that Autologous transplantation of bone marrow mesenchymal stem cell serves as an immuno-competent tool to fasten hair growth in patient with alopecia and old age.

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