Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
**eTable. Primers Used for Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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</thead>
<tbody>
<tr>
<td>(\beta)-actin</td>
<td>5’CATGTACGTTGCTATCCAGGC3’</td>
<td>5’CTCCTTAATGTCACGCACGAT3’</td>
</tr>
<tr>
<td>SCF</td>
<td>5’ACTGAAGGGATCTGCAGGAA3’</td>
<td>5’TCTTTTCACGCACTCCACAAG3’</td>
</tr>
<tr>
<td>bFGF</td>
<td>5’AGAGCGACCCTCACATCAAG3’</td>
<td>5’ACTGCCAGTTTGTTTTCAGT3’</td>
</tr>
</tbody>
</table>
eAppendix. Methodology: In Vitro Experiments

1. Flow cytometry:

Single cells suspension obtained from epidermal graft (ECS) and hair follicles (FCS) as described in the suspension preparation section was used for the flow cytometry. Cells were pellet down by centrifugation at 1000 rpm for 5 minutes. Cells were then permeabilized with triton X-100 for 10 minutes followed by washing with FACS buffer and blocking in bovine serum albumin (BSA) for 30 minutes. Next, cells were incubated with mouse monoclonal primary antibodies HMB-45 (homatropine methylbromide 45, Abcam, UK) and OCT4 (octamer-binding transcription factor 4, Santa Cruz Biotechnology, Inc., USA) for 2 hours at room temperature. Cells were washed three times with FACS buffer and then incubated with FITC labelled anti-mouse secondary antibodies (Santa Cruz Biotechnology, Inc., USA) for 1 hour at room temperature. Cells were analyzed using a FACScan (BD Biosciences, USA).

2. Gene expression studies at transcriptional level:

For gene expression studies at transcriptional level, total cellular RNA was isolated followed by cDNA synthesis and amplification of cDNA by real time PCR. The following protocol was followed:

   a) RNA Isolation and cDNA Synthesis:

   Total cellular RNA was extracted from the cells using TRI reagent (Sigma Aldrich, USA) by following manufacturers’ guidelines. The quantity and quality of isolated RNA was determined spectrophotometrically by measuring absorbance at 260 nm and 280 nm in Nanodrop (Thermo Fisher Scientific, USA) and it was immediately subjected to cDNA synthesis.
First strand cDNA was synthesized from 2 µg of total cellular RNA using random hexamer primers and Moloney murine leukemia virus (M-MuLV) reverse transcriptase in a 20 µl reaction volume. Reagents were obtained from RevertAidTM first strand cDNA synthesis kit (MBI Fermentas, Germany).

b) Quantitative Real Time-polymerase chain reaction (qRT-PCR):

qRT-PCR was performed using the SYBR Green master mix on Real-Time PCR System (LightCycler 480, Roche Molecular Diagnostics, Inc., USA) to determine the transcriptional expression of β-actin, SCF and bFGF. cDNA of each sample was amplified using specific primers for SCF, bFGF and β-actin. The gene specific primers were obtained from Sigma Aldrich, USA (eTable).
eFigure 1. Comparison of Extent of Repigmentation in ECS+FCS Over ECS at Each Follow-up Visit. Positive and negative rankings here implies greater and lesser repigmentation scores respectively (each score = 25%) and tie implies no difference in repigmentation compared to paired patch in a patient at a particular follow up visit. Superior repigmentation in ECS+FCS increased from 13 patches showing better repigmentation score at 4th week to 18 patches showing better repigmentation score at 16th week. Repigmentation score of two patients was inferior in ECS+FCS side compared to ECS side throughout the study.
**eFigure 2.** Extent of Repigmentation at Week 16 Follow-up.

ECS+FCS, combined epidermal and follicular cell suspension; ECS, epidermal cell suspension.
**eFigure 3:** Flow Cytometry of Cell Suspensions. Epidermal cell suspension (ECS), follicular cell suspension (FCS) and combined epidermal and follicular cell suspension (ECS+FCS) were analyzed with flow cytometry using melanocyte marker, HMB45 and stem cell marker, OCT 4: (A) ECS-NEGATIVE CONTROL, (B) ECS- HMB45, (C) ECS-OCT-4 (D) FCS-NEGATIVE CONTROL (E) FCS-HMB45, (F) FCS-OCT-4, (G) ECS+FCS-HMB45, (H) ECS+FCS-OCT-4.
**eFigure 4.** Quantitative Real Time Expression of Stem Cell Factor (SCF) in the epidermal cell suspension (ECS), combined epidermal and follicular cell suspension (ECS+FCS) and follicular cell suspension (FCS). Data is presented as mean ± SD. Statistical significance is shown by *p<0.05 vs. ECS.
eFigure 5. Quantitative real time expression of basic fibroblast growth factor (bFGF) in the epidermal cell suspension (ECS), combined epidermal and follicular cell suspension (ECS+FCS) and follicular cell suspension (FCS). Data is presented as mean ± SD. Statistical significance is shown by *p<0.05 vs. ECS.