

## Enrichment of putative human epidermal stem cells based on cell size and collagen type IV adhesiveness

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The enrichment and identification of human epidermal stem cells (EpSCs) is of paramount importance for both basic research and clinical application. Though several approaches for EpSCs enrichment have been established, enriching a pure population of viable EpSCs is still a challenging task. Improved approach is worth developing to enhance the purity and viability of EpSCs. Here we report that cell size combined with collagen type IV adhesiveness can be used as a improved approach to enrich human EpSCs for high purity and viability. We separated the rapidly adherent keratinocytes into three populations ranged in size from 5 to 7  $\mu$ m (population A), 7 to 9  $\mu$ m (population B) and = 9  $\mu$ m (population C) in diameter and found human putative EpSCs could be further enriched in population A with the smallest size. Among these three populations, population A displayed the highest density of  $\beta$ 1 integrin receptor, contained the highest percentage of cells in G0/G1 phase, showed the highest nuclear to cytoplasmic ratio, and possessed the highest colony formation efficiency (CFE). When injected into murine blastocysts, these cells participated in multi-tissue formation. More significantly, compared with previous approach that sorted putative EpSCs based on  $\beta$ 1 integrin antibody staining, the viability of the EpSCs enriched by the improved approach was significantly enhanced. Our results provide a putative strategy for human EpSCs enrichment, and encourage further study of the role of the cell size in stem cell biology.

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