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A simple culture method for epithelial stem cells derived from human hair follicle

Research Article

Abu Bakar Mohd Hilmi[1], Ahmad S. Halim[1,*], Norhayati Mohd Noor[1], Chin K. Lim[1,2], Zamzuri Idris[3], Abdullah Pohchi[4], Hassan Asma[5], Shaik Farid Abdull Wahab[6], Stephan Tiede[7], Ralf Paus[7]

[1]*Reconstructive Sciences Unit, Universiti Sains Malaysia, 16150 Kelantan, Malaysia*

[2]*Faculty of Dentistry, Universiti Teknologi MARA, 40450 Selangor, Malaysia*

[3]*Department of Neurosciences, Universiti Sains Malaysia, 16150 Kelantan, Malaysia*

[4]*School of Dental Sciences, Universiti Sains Malaysia, 16150 Kelantan, Malaysia*

[5]*Department of Anatomy, Universiti Sains Malaysia, 16150 Kelantan, Malaysia*

[6]*Department of Accident and Emergency, Universiti Sains Malaysia, 16150 Kelantan, Malaysia*

[7]*Department of Dermatology, University of Lübeck, 23538 Lübeck, Germany*

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Abstract: The challenge arises among researchers when hair follicle stem cells (HFSCs) derived from a human hair follicle remain poorly expanded in defined culture medium. In this study, we isolated the HFSCs and they became confluent after 10 days of cultivation. Comparing the viability of HFSCs cultured in defined keratinocytes serum free medium (KSFM) in a coated plate and CnT07 medium in an uncoated plate, the number of HFSCs cultured in CnT07 was significantly higher at days 2, 4, 6 and 8 ($P=0.004$). The population doubling time of HFSCs was 21.48±0.44 hours in non-coated plates with CnT07 and 30.73±0.75 hours in coated plates with KSFM. Our primary HFSC cultures were positive for CD200 and K15 with brownish color. Flow cytometry analysis showed that the percentage of HFSCs expressing CD200 and K15 were 65.20±3.16 and 72.07±6.62 respectively. After reaching 100% confluence, the HFSCs were differentiated into an epidermal layer *in vitro* using CnT02-3D defined media. HFSCs were differentiated into an epidermal layer after 2 weeks of induction. Involucrin- and K6-positive cells were detected in the differentiated epidermal layer. This method is a simple technique for HFSC isolation and has a lower cost of processing and labor, and it represents a promising tool for skin tissue engineering.

Keywords: *Xeno-free • Human hair follicle stem cells • Differentiation in vitro*

1. Introduction

Hair follicle stem cells (HFSCs) reside in a structure within the outer root sheath of the hair follicle known as the bulge [1,2]. In the bulge, the cells exist in an undifferentiated state, and their normal role is to generate the hair follicle (HF) and regrow the hair shaft. During the hair growth cycle, HFSCs are localized in anagen at the site of the arrector pili muscle and rapidly proliferating progenitor cells in the bulb generate the hair shaft and its surrounding inner root sheath [2]. If skin injury occurs, HFSCs can contribute to repair of

* *E-mail: ashalim@kb.usm.my*