

**UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR**

**INVOLVEMENT OF MAPK/ERK AND AKT SIGNALING
PATHWAYS IN THE PROLIFERATION OF KERATINOCYTES
COCULTURED WITH ADIPOSE-DERIVED STEM CELLS (AZSS)-
POROUS CHITOSAN SCAFFOLD (PSC)**

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A	PROJECT DETAILS
i	Title of Research: Involvement of MAPK/ ERK and Akt signaling pathways in the proliferation of keratinocytes cocultured with adipose-derived stem cells (ACSS)-porous chitosan scaffold (PCS).
ii	Account Number: 1001/PPSP/813058
iii	Name of Research Leader: PROF DR AHMAD SUKARI HALIM
iv	Name of Co-Researcher: <ol style="list-style-type: none">1. Prof Dr Nik Soriani Yaacob2. Prof Madya Dr. Mohamed Saifulaman bin Mohamed Said3. Dr Aravazhi Ananda Dorai4. Dr Lim Chin Keong
v	Duration of this research: <ol style="list-style-type: none">a) Start Date : 15th July 2012b) Completion Date : 14th July 2015c) Duration : 36 monthsd) Revised Date (if any) : 15th July 2016

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B ABSTRACT OF RESEARCH**MODEL KULTUR DWI-RUANG UNTUK KAJIAN SEL STEM DARI TISU LEMAK DAN KITOSAN BERLIANG TERHADAP PERTUMBUHAN KERATINOSIT DARI MANUSIA
ABSTRAK**

Bahan yang dirembeskan oleh sel stem dari lemak berpotensi mempunyai kesan parakrin yang berpotensi dalam mempercepatkan pertumbuhan keratinosit melalui kaedah pengkulturan secara bersama. Kajian ini dijalankan untuk menilai kesan tidak langsung sel stem dari lemak dan kitosan berliang terhadap pertumbuhan keratinosit melalui parakrin. Tisu lemak dan epidermis yang diperolehi dari manusia dikultur dan dikesan menggunakan penanda spesifik. Pengagihan dan pertumbuhan sel stem pada kitosan berliang dinilai melalui mikroskop imbasan elektron, "live/dead" dan Alamar blue. Kumpulan dwi-kultur telah dibahagikan kepada dua; kultur stem sel dari lemak dan kombinasi sel stem dari lemak dan kitosan berliang. Pertumbuhan keratinosit semasa pengkulturan bersama dianalisis dengan ujian Alamar blue pada 24 jam dan 72 jam pengkulturan bersama manakala kepekatan faktor pertumbuhan epidermal (EGF) dikesan selepas 72 jam. Kultur sel-sel stem telah dihasilkan dari tisu lemak didapati mengekspresi CD29, CD73, CD90, CD105 pada kadar yang tinggi manakala CD34 pula rendah. Ujian imunokimia juga menunjukkan kultur keratinosit mengekspresi involucrin dan cytokeratin 6. Peratus pertumbuhan sel stem dari lemak di dalam kitosan berliang adalah lebih daripada 80% disertakan dengan ciri-ciri berbentuk gelendong dan penghidupan yang bagus. Selepas 72 jam pengkulturan bersama, didapati peratusan keratinosit dalam kumpulan pengkulturan bersama telah dipercepatkan disebabkan oleh media kultur yang digunakan. Hasil dari ujian ELISA menunjukkan tiada rembesan EGF dari sel stem lemak. Kajian ini telah menunjukkan bahawa melalui model kultur dwi-ruang, pertumbuhan keratinosit tidak dirangsang melalui rembesan EGF dari sel stem lemak. Ianya berkemungkinan disebabkan oleh faktor pertumbuhan lain atau mekanisma lain.

A DUAL-CHAMBER COCULTURE MODEL FOR THE STUDY OF HUMAN ADIPOSE-DERIVED STEM CELLS AND POROUS CHITOSAN SCAFFOLD (PCS) ON THE GROWTH OF HUMAN KERATINOCYTES**ABSTRACT**

Secretory factors of adipose-derived stem cells have paracrine effects that may have potential in accelerating proliferation of keratinocytes by coculturing both of the cells. This study was conducted to evaluate the indirect effects of ASCs-PCS on the growth of keratinocytes by paracrine activities. Human adipose-derived stem cells and human epidermal keratinocytes were isolated and verified with their specific markers. Distribution and proliferation of ASCs on porous chitosan scaffold were assessed by scanning electron microscope, live/dead assay and Alamar blue assay. Coculture groups were divided into two groups; culture ASCs and culture of ASCs-PCS. Proliferation of keratinocytes in the coculture wells were analysed by Alamar blue assay at 24 hours and 72 hours of coculture while the concentration human epidermal growth factor in culture supernatant were quantified at 72 hours of coculture. Adipose-derived stem cells culture have been established and displayed high expression of CD29, CD73, CD90, CD105 and low expression of CD34. Keratinocytes culture expressed involucrin and cytokeratin 6 as demonstrated by immunocytochemistry. Percentage of proliferation of ASCs within PCS was more than 80% accompanied with spindle-shaped features and good viability. After 72 hours of coculture, the percentage of nHEK in the coculture group was increased due to growth medium. ELISA assay revealed no secretion of EGF from ASCs. This study demonstrated *via* dual-chamber coculture model, human-derived ASCs and PCS showed no growth stimulatory effect towards the growth of keratinocytes *via* the tested growth factor, EGF. The proliferation of nHEK in the coculture wells possibly due to other growth factor or other mechanism.