

Volume 6, No. 1 (Supplement)

June 2011

ISSN 1823-2140

The
National University
with an
INTERNATIONAL REACH

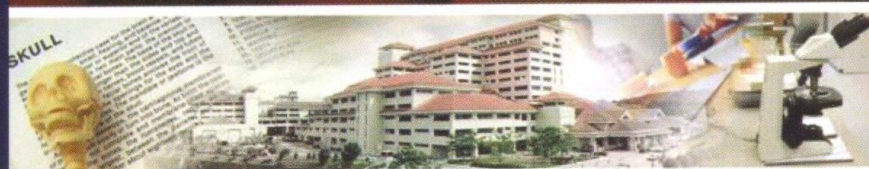


UNIVERSITI
KEBANGSAAN
MALAYSIA
National University of Malaysia

MEDICINE & Health

The Official Journal of The Faculty of Medicine UKM

7th
Malaysia Indonesia Brunei
Medical Sciences Conference
"TOWARDS A HOLISTIC AND INTEGRATIVE
APPROACH IN HEALTHCARE"



22nd - 24th July 2011

Equatorial Hotel, Bangi, Selangor,
MALAYSIA

officiated by

Y.B Datuk Rosnah Haji Abdul Rashid Shirlin
Deputy Minister of Health Malaysia

Organised by



HUMAN KERATINOCYTES AND FIBROBLAST GENE EXPRESSIONS WERE MAINTAINED EVEN WHEN TRYPSINIZED WITH RECOMBINANT TRYPSIN: TOWARDS CLINICAL APPLICATION

Khairul AK¹, Manira M¹, Seet WT¹, Ahmad Irfan AW¹, Ng MH¹, Chua KH^{1,2}, Aminuddin BS^{1,3}, Ruszymah BH^{1,2}

¹Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

²Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

³Ear, Nose and Throat Consultant Clinic, Ampang Puteri Specialist Hospital, Ampang, Malaysia

Background:

Animal derivative free trypsin, is a current Good Manufacturing Practice (cGMP) requirement to be used in human treatment. Performance of two different types of trypsin in trypsinization process of keratinocytes and fibroblast cells were studied to evaluate their activity level.

Materials and Methods:

Human skin was digested in 0.6% Collagenase Type I for six hours to isolate the fibroblast. This was followed by trypsinization of epidermal layer using animal derived trypsin, Trypsin EDTA (TE) and recombinant trypsin, TrypLE Select (TS). The cells were cultured until passage 2 and then trypsinized using either TE or TS. Gene expression of Collagen type III, Cytokeratin 10 and Cytokeratin 14 were quantitatively analyzed by using RT-PCR and further confirmed by immunocytochemistry staining.

Results:

Cells trypsinized using both trypsins positively expressed the specific gene of interest. TS showed higher gene expression level for both Cytokeratin 10 (TE: 0.012 ± 0.065 ; TS: 0.396 ± 0.085) and Cytokeratin 14 (TE: 0.160 ± 0.076 ; TS: 0.321 ± 0.101) compared to TE for keratinocytes, but they were all not significant. Expression of Collagen type III genes in TS was slightly lower compared to TE for dermal fibroblast (TE: 0.024 ± 0.012 ; TS: 0.015 ± 0.001) but was also not significant. Immunocytochemistry staining supported the finding and showed that keratinocytes was positively stained with Cytokeratin 10 and Cytokeratin 14 antibodies. Collagen type 1 was expressed in fibroblast.

Conclusion:

This demonstrated that dermal fibroblast and keratinocytes maintained their characteristics even when trypsinized with recombinant trypsin. Therefore, recombinant trypsin can be used as an alternative to animal derived trypsin for clinical application.

Keywords:

tissue engineering, recombinant Trypsin, cGMP, fibroblast, keratinocytes