

Measurement of NF- κ B as an indicator of stress in skin cells in 2D culture and a 3D reconstructed skin model

T. Sun¹, J.W. Haycock¹, M. Szabo¹, R.P. Hill¹, and S. MacNeil^{1,2}

¹Department of Engineering Materials and ²Section of Medicine, University of Sheffield, U.K.

INTRODUCTION: NF- κ B is a DNA-binding transcription factor that plays an essential role in the regulation of many genes, especially those involved in stress, immune and inflammatory responses. Human skin keratinocytes and fibroblasts, in common with many other cell types in the body, respond to common proinflammatory cytokines such as TNF- α with a rapid activation of NF- κ B. However, various stimuli including viral infection, lipopolysaccharide, UV irradiation, shear stress and intracellular pH can also activate NF- κ B. The aim of the present study was to develop an immunofluorescence method for measuring acute NF- κ B activity in keratinocytes and fibroblasts in normal and reconstructed human skin, based on a similar approach for measurement of cells in monolayers. We also investigated the robustness of the technique by exposing cells to TNF- α , pH and a depletion of culture medium buffering potential.

METHODS: Culture of human dermal fibroblasts, preparation of human skin and reconstructed human skin were as previously described [1]. Cells were stimulated with TNF- α , incubated with pH-adjusted medium or incubated in an unbuffered atmosphere to investigate inflammatory, pH and carbon dioxide tension as indices of stress. Immunofluorescent staining of p65/NF- κ B was performed as reported previously [1,2]. Fluorescence micrographs of immunolabelled samples were taken using a Leica DM-IRB inverted fluorescent microscope using epifluorescent illumination at λ_{ex} = 495nm, λ_{em} = 515nm (for FITC / NF- κ B visualization) and λ_{ex} = 358nm, λ_{em} = 461nm (for DAPI / nuclei visualization). NF- κ B activation was calculated based on nuclear versus cytoplasmic localisation using Improvisation Openlab v3.0.2 [2]. Normal and reconstructed human skin were treated as above and then processed for cryosectioning (5 μ m), followed by analysis of NF- κ B using the above immunolabelling approach.

RESULTS: Incubation of fibroblast cells with TNF- α rapidly activated NF- κ B. This was observed by immunofluorescence microscopy as rapid translocation from the cytoplasm to the nucleus. Incubation of cells for 60 minutes in medium with pH adjusted from 6.3 to 8.5 also

activated NF- κ B, in comparison to cells maintained at a physiological pH of 7.3 (Figure 1). Fibroblasts were also depleted of buffered culture medium by incubation in 21% O₂ / 79% N₂. Similarly, an increase in NF- κ B activation was found with time, correlating with medium acidosis. NF- κ B analysis by immunofluorescent microscopy was then used for the analysis of normal and reconstructed human skin containing keratinocytes and fibroblasts. A similar activation of NF- κ B to TNF- α , pH and unbuffered conditions for both cell types was observed. However, it was also possible to monitor the relative activation of the two cells types based on cellular position in the tissue.

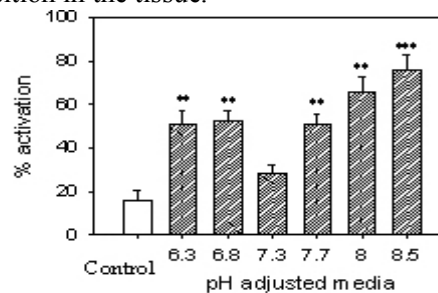


Figure 1. Effect of pH on human fibroblast NF- κ B activation

DISCUSSION & CONCLUSIONS: We have developed an immunofluorescent technique for measuring NF- κ B activity, initially used for analysis of cells in 2D, for analysis of cells in a 3D skin model. Using this method, inflammatory stimuli, pH and culture medium buffering potential were found to activate NF- κ B in cultured fibroblast monolayers, and keratinocytes and fibroblasts in normal human and reconstructed skin. This study highlights the sensitivity of using NF- κ B transcription factor activation as an indicator of inflammatory, pH or environmental stress. Application of this technique for measuring NF- κ B of cells contained in tissues will have considerable value for the analysis of cellular stress in tissue engineering applications.

REFERENCES: ¹ Chakrabarty KH et al. (1999) Brit J Dermatol. 141, 811. ² Moustafa M et al. (2002) J Invest Dermatol. 119, 124.

ACKNOWLEDGEMENTS: We would like to thank the EPSRC for financial support.