

Effects of protein coated substrates on keratinocyte monolayer wound closure

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INTRODUCTION

In order to optimise skin repair systems it is necessary to develop a thorough understanding of the wound healing process and the way in which keratinocytes interact with their surrounding physiochemical environment during reepithelialisation. The role of extracellular matrix proteins, integrins and growth factors in the migration and behaviour of these cells will have to be well understood. This study uses the well established scratch assay to examine the wound closure response of keratinocyte HaCaT cells to different extracellular matrix proteins; fibronectin, laminin and collagen type I.

METHODS

Proteins were coated onto the surface of 25cm² flasks into which HaCaT cells were seeded and cultured for 48 hours. Confluent HaCaT cell monolayers were 'wounded' and the rate of wound closure was measured over a 10 hour period.

RESULTS AND DISCUSSION

Cells on laminin showed only 35% closure at 6 hours compared to 53% with control, 51% on fibronectin and 53% on collagen type I. At 8 hours, these differences increased to 61% closure on laminin

compared to 87% for control, 84% on bronectin and 83% on collagen type I.

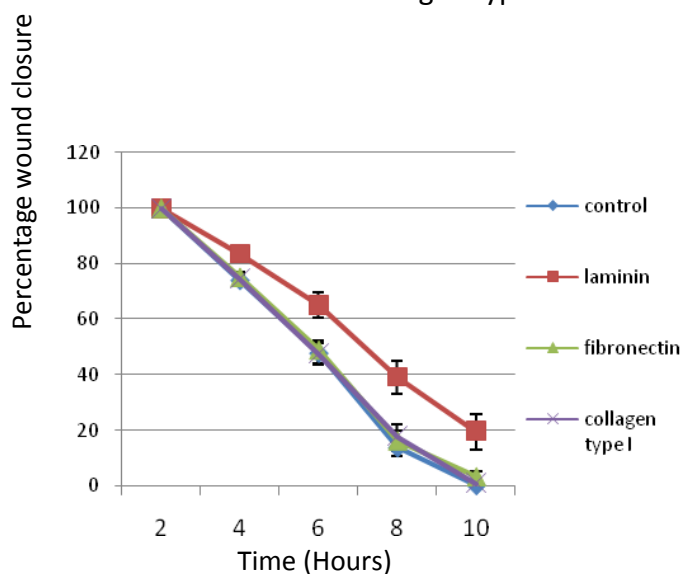


Figure shows the linear relationship between percentage wound closure and time. Standard error bars are shown (n=15).

Laminin coated surfaces appear to cause a reduction in the rate of wound closure. Future work will aim to determine how cellular responses to laminin are modified in a model wound by cytokines such as the TGF β isoforms, particularly TGF β 3.

CONCLUSION

We demonstrate that laminin is an important rate limiting factor in the closure of monolayer wounds with respect to HaCaT cells.