Effects of protein coated substrates on keratinocyte monolayer wound closure

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Introduction

Tissue engineering will play a major role in the future developments in treatment of skin wounds. 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 ECM proteins, integrins and growth factors in the migration and behaviour of these cells will have to be well understood.

The scratch assay has been commonly used to assess cell behaviour in response to "wounding" of cultured cell monolayers. This study uses the scratch assay to examine the wound closure response of keratinocyte HaCaT cells to different extracellular matrix proteins; fibronectin, laminin and collagen type I with the eventual aim of determining how cellular responses to these ECM molecules are modified in a model wound by cytokines known to enhance wound repair.

Key words; wound healing, scratch assay, cell migration, laminin.

Aims and Objectives

To determine how protein coated substrates effect the 'healing' of HaCaT cell monolayer wounds.

Methods

The proteins laminin, fibronectin and collagen type I were coated onto the surface of 25cm^2 flasks. HaCaT cells were cultured with each protein for 48 hours until confluence was reached. Monolayers were then "wounded" by scratching the surface of the flask using a 1ml pipette. Wound sites were imaged using Visicapture software every 2 hours. Wound closure was measured at each time point using Image J software.

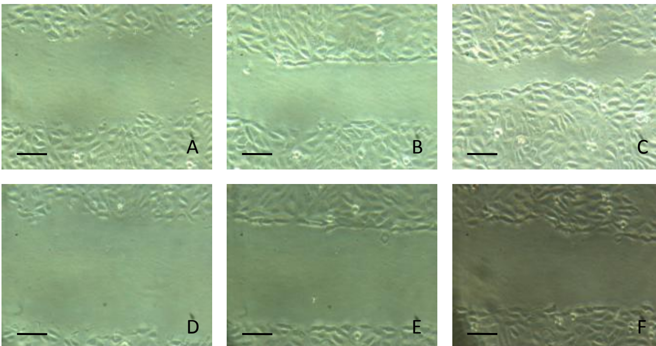

Figure 1: HaCaT cells under control conditions at 2, 4 and 6 hours (A-C respectively); HaCaT cells plated on Laminin (D-F) at 2, 4 and 6 hours post wounding. Scale bar = approx. 100µm.

Results and Discussion

Raw measurements (µm) of the wound at each time point were normalised and average data was plotted as shown in Fig 2. Results show significant differences between closure of a wounded monolayer on a laminin coated substrate when compared to the control.

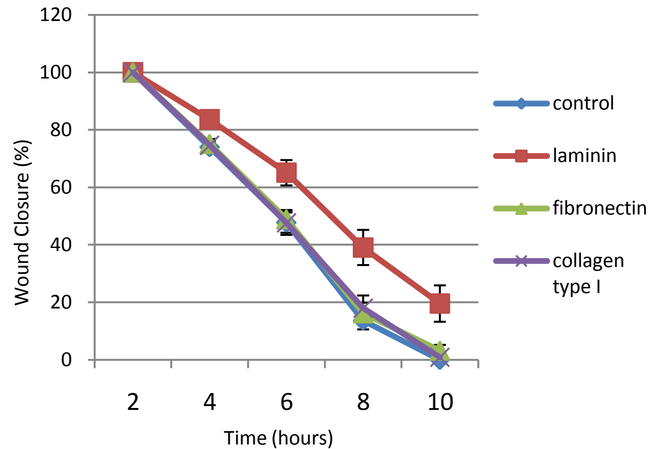

Figure 2 shows the linear relationship between percentage wound closure and time. Standard error bars are shown (n=15).

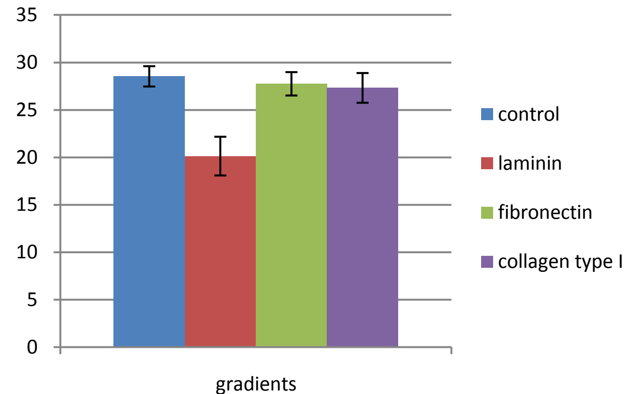

Figure 3: Bar chart to represent average gradient values for each protein. Standard error bars are shown.

HaCaT 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 fibronectin and 83% on collagen type I. Laminin coated surfaces appear to cause a reduction in the rate of wound closure.

Trend lines were added for each data set. The equation $y=mx+c$ and R^2 value for each trend line were used to determine the gradient (value of 'm') and reliability of the trend line. The gradients were used to compare the rate of wound closure for each protein. These mean gradient values are shown in Fig 3. Average R^2 values for the control, laminin, fibronectin and collagen type I were 0.98, 0.96, 0.98 and 0.98 respectively.

Conclusion

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