The role of Cx43-mediated signalling in diabetic and non diabetic wound healing events

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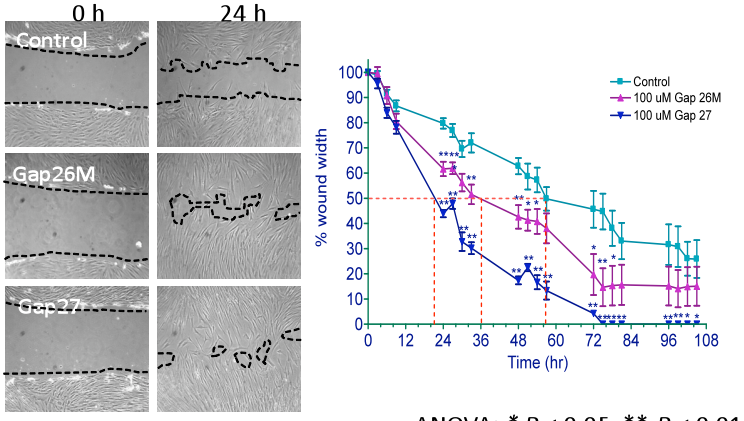
The problem: Currently in the U.K. 2.6 million people suffer from type II diabetes forming considerable and increasing burdens on healthcare resources. A major complication is delayed wound healing and ulceration. During disease progression, vascular complications ensue, which affects the skin and contributes to altered wound closure rates as the increased circulating glucose and insulin levels impact on cellular responses in the wound bed including cell migration rates, deposition of extracellular matrix and inflammatory responses. This can result in chronic non-healing wounds with leg and foot ulceration and lower extremity amputations. Following 20 weeks of standard care 67% of diabetic foot ulcers remain unhealed. Conventional treatments (debridement, compression bandaging and antibiotics to control infection) are labour intensive, costly and relatively ineffective, whilst pharmacological approaches (e.g. growth factors) have yet to show clinical utility. Thus defining new therapeutic agents that can be directly incorporated onto a wound bed in a topical form could provide significant improvements in patient care and reduce NHS costs.

The gap junction protein connexin43 (Cx43) exhibits differential remodelling in 'normal' and 'chronic' wounds [3], and small peptides targeted to this protein (CMPs) improve wound closure rates in *ex vivo* and *in vitro* organotypic skin model systems and suggest a novel therapeutic route [1,2]. The mechanism underlying peptide action remains unresolved and may include changes in rates and or directionality of cell movement.



Wright *et al.*, 2009

Figure 1: Scrape wounds were introduced into confluent monolayers of human dermal fibroblasts and 'wound closure' rates monitored at 6 hourly intervals in the presence or absence of CMP. Significant increase in closure rates occur in the presence of CMP.

Experiments: In the experiments, we can compare cell dynamics during cell adhesion and migration under differing cellular conditions in the presence or absence of the peptides. Two 2D and 3D cultures of keratinocytes and fibroblasts will be used as required and a 'scrape wound' introduced (Figure 1). Cell migration into the 'gap' will be monitored by time-lapse video microscopy and data recorded. This will enable the extent to which cells within a migrating population behave

independently or in concert (in terms of directionality and rate of movement, cell divisions, cell shape, organisation of cytoskeleton and cell-cell junctions). Analysis of the effects of CMP upon cell population responses to interaction with various extracellular matrix-coated substrates will test the interactions of connexins with adhesion and migration signalling pathways.

Mathematical modelling: We propose to assess modes of cell movement and time taken for 50% closure of the 'wound gap' by determining spatio-temporal differences in cellular events. An ideal outcome from the workshop is the development of an accurate and validated description of the wound healing process – incorporating key factors, such as individual cell movement, cell-cell interactions, cell divisions, protein production and utilization. We are aware of some previous theoretical attempts that use an individual based modelling approach (see for example [3-5]) but these lack any details of cell-cell protein signalling, cell-matrix interactions or wound biomechanics in general. Our empirical data can help focus the modelling to the key protein and cellular interactions involved in our empirical systems and be used to parameterize and validate the theoretical descriptions of the cell migration assays. The resulting models can then be used to provide a platform for quantifying the observed events, providing cellular information on the impact of remodelling connexin signalling and adhesion events on cell migration and motility responses, and also be used as an additional tool for opitimization of the healing process in diabetic wounds.

References

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