Development of a Cell-based Diabetic Wound Assay

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Summary — Chronic wounds require prolonged healthcare and adversely affect the quality of life of patients. They are particularly prominent in patients with diabetes, and their relative numbers are set to increase with the rise of diabetes within our population. Research is still needed to understand the factors leading to such wounds, to understand why they persist for such long periods of time, and also to develop new and efficacious treatment strategies. One problem facing this research is a lack of adequate animal models, as the current models do not truly reflect the human condition and often lead to much animal suffering. Hence, over the past four years, our group has been trying to develop a human-based in vitro diabetic wound model, which could be used as a high-throughput screening system to pre-screen potential chronic diabetic wound healing agents and to reduce unnecessary animal pain and suffering. To this end, we have isolated healthy and diseased skin fibroblasts from patient tissue biopsies. Crucially, to create a cell reporter system that can be widely used in the future, the cells were immortalised in order to escape senescence. By using microarray analysis, gene expression pattern differences have been identified between healthy and diseased cells, and disease-specific ‘reporter’ genes have been selected for further studies. The promoters of these reporter genes have been coupled to fluorescent reporter constructs and inserted back into the diseased fibroblasts, so that we now have proof-of-concept for a real-time diabetic reporter system for future exploitation.

Key words: chronic wound model, diabetic ulcer, fibroblasts, wound healing.

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Introduction

Unfortunately, chronic wounds are relatively common and serious, and occur more frequently in diabetic and aged individuals. Due to an expanding ageing population (by 2020, 20% of the UK population will be over 65 years old), the treatment of chronic wounds will consume an increasingly large amount of the resources and time of healthcare providers (1).

As is well recognised, ageing affects the structure and appearance of our skin, evidenced by a thinning of the tissue which is associated with dryness and wrinkling (2). However, it is only on histological analysis that it is possible to fully appreciate the underlying process, which is fundamentally due to changes in the extracellular matrix (loss of collagen, elastin and glycosaminoglycans and an increased amount of cross-linking). These changes, together with a degree of dermo-epidermal flattening, mean that the skin does not respond to insults in the way that it should, so it tends to break down more easily. One of the main issues with ageing of the skin is that it leads to problems with respect to wound healing. This includes a protracted, non-resolving inflammatory response, the inability of fibroblasts to remodel any connective tissue they have produced, and the inability to re-epithelialise the wound. Under such circumstances, the wound does not develop an adequate cover to resist the invasion by microorganisms. Impaired angiogenesis is another factor which contributes to poor wound healing — new blood vessel formation is crucial, as the tissue must have an adequate supply of oxygen and nutrients. Ultimately, all this leads to reduced wound contraction/reorganisation and dysfunctional wound closure. If, however, wounds do heal, they may breakdown again very easily. Unfortunately, this results in chronic wounds, such as diabetic foot ulcers, chronic venous leg ulcers and pressure ulcers.

Chronic wounds in general cost the UK National Health Service (NHS) over £1-billion per annum, and the cost of treating these wounds is predicted to increase substantially as the population ages (1). There are approximately 2-million diabetes sufferers in the UK, and 200-million worldwide. Type II diabetes is associated with impaired wound healing, and 15% of diabetic patients will develop foot ulcers. Hospital admissions resulting from these wounds account for 6% of hospitalisations (in the US) and cost about £5,000–11,000 per case. If amputation ensues, the majority of patients will die within five years.