Wound Repair and Regeneration

TECHNICAL ARTICLE

Fluorescent cell tracer dye permits real-time assessment of re-epithelialization in a serum-free ex vivo human skin wound assay

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ABSTRACT

Ex vivo wounded human skin organ culture is an invaluable tool for translationally relevant preclinical wound healing research. However, studies incorporating this system are still underutilized within the field because of the low throughput of histological analysis required for downstream assessment. In this study, we use intravital fluorescent dye to lineage trace epidermal cells, demonstrating that wound re-epithelialization of human ex vivo wounds occurs consistent with an extending shield mechanism of collective migration. Moreover, we also report a relatively simple method to investigate global epithelial closure of explants in culture using daily fluorescent dye treatment and en face imaging. This study is the first to quantify healing of ex vivo wounds in a longitudinal manner, providing global assessments for re-epithelialization and tissue contraction. We show that this approach can identify alterations to healing with a known healing promoter. This methodological study highlights the utility of human ex vivo wounds in enhancing our understanding of mechanisms of human skin repair and in evaluating novel therapies to improve healing outcome.

Experimentally wounded human skin organ culture has proven to be an invaluable tool for translationally relevant preclinical research for the past 20 years.[1] Many different groups have now developed similar approaches to understand basic mechanisms of human wound healing and for identifying novel candidate wound healing promoters.[2–8] In addition, assays using porcine skin ex vivo have been established[9–11] because of its anatomical similarity and strong concordance with human healing.[12] Reconstituted skin cultures (also known as organotypic or skin equivalent cultures), which comprise keratinocytes seeded onto a fibroblast-containing collagen gel, are also available to model human wounds.[13–15] Both skin explant and skin equivalent approaches display epithelial migration across the underlying matrix to heal the wound, although ex vivo skin is thought to mimic the in vivo situation more closely.[16,17] Injury to artificial skin equivalent cultures initiates collective cell migration and wounds re-epithelialize through the formation of an extending shield,[14] although resolving whether this same mechanism is conserved in the much more complex environment found in human skin is yet to be addressed.

The accurate measurement of wound re-epithelialization in 3D experimental models has proved challenging as a result of high workload required to serially section the entire tissue, which is essential to ensure that the center of each wound is assessed. In addition, standard histology only provides a snapshot through the wound, and so, it is unclear how consistent the evaluated region might be to other areas within the wound.

Macroscopic assessment of wound closure forms the primary assessment for progression of healing in chronic wounds, given that biopsies may further jeopardize healing.[18] Thus, current ex vivo models typically examine wound healing in an entirely different plane to "real-life" observations made in the clinic.

Several recent studies have reported imaging the surface of ex vivo and organotypic wound cultures, using a range of different methods, which exploit differing spectroscopic properties of the various wound cell types. These include the use of infra-red and Raman spectroscopy,[19] measuring ultraviolet (UV) auto-fluorescence,[8] magnetic resonance imaging,[20] and revealing wound topography using fringe projection.[21] In addition, the recent article in *Wound Repair and Regeneration* by Glinos et al.[22] describes optical coherence tomography (OCT) as a method to differentiate newly formed epithelial tissue from open wound in human wounds ex vivo and provides the highest clarity of imaging reported thus far.

In our recent experiments, we have established a serum-free ex vivo human partial thickness wound model (see Methods for full details). We have been examining the green

