Keratinocytes cultured from human chronic wound specimens demonstrate delayed wound closure and differences in apoptosis in *in vitro* scratch assay

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Abstract

nd healing is a critically important physiologic ess which restores the normal epidermal barrier tion of the skin after injury. Chronic wounds that e failed to heal affect approximately 6.5 million ple in the US with a prevalence of 1% and costs nated at $25 billion per year. The purpose of this y was to utilize a keratinocyte scratch assay to stigate molecular mechanisms of human wound ing.

Introduction

keratinocytes are programmed to maintain skin integrity. Wounding es keratinocyte migration and activation[1,2]. Keratinocyte function is to normal wound healing,[3] and thus it makes sense to investigate nces in function between keratinocytes harvested from normal skin those hronic wound specimens. Defective keratinocyte function is also thought a crucial role in the chronic inflammatory skin disease hidradenitis ativa (HS)[4].

rpose of the current study was to demonstrate that primary cultured ocytes isolated from skin of patients with chronic wounds and HS strate differences in migration and viability compared to keratinocytes d from normal human skin.

Methods

search was conducted through the Wound Etiology and Healing (WE-tudy, a biospecimen and data repository designed for studying chronic and hidradenitis suppurativa, approved by the George Washington ity Institutional Review Board (041408).

epidermal keratinocytes were cultured at $2x10^5$ cells/well in a 6 well Eppendorf, NY). Cells were allowed to reach 80% confluence before a cratch was made according to established methods[5] with a sterile 1 mL tip. Individual wells were photographed at pre-scratch, 0, 24, 48, 72, and rs using an inverted phase contrast microscope at 50x magnification)M IRB, Leica Microsystems, Germany). Total scratch surface area was ed using ImageJ software (National Institutes of Health, Bethesda, MD).

bility was assessed using the ReadyProbes® Cell Viability Imaging Kit (Life logies, ThermoFisher, CA). Viable cells were detected with a Nuc-Blue through a standard DAPI filter. Non-viable cells were detected with a een reagent through a FITC/GFP filter. Images were captured using cence microscope at 100x (Nikon Eclipse TE300, Nikon Instruments, , NY) and merged to get a composite image of viable/non-viable cells at rs. Two representative images from each well were assessed by two ndent observers blinded as to clinical group.

Results

Scratch Assay

Normal keratinocytes demonstrate faster closure of the scratch than chronic wound keratinocytes (Figure 1).

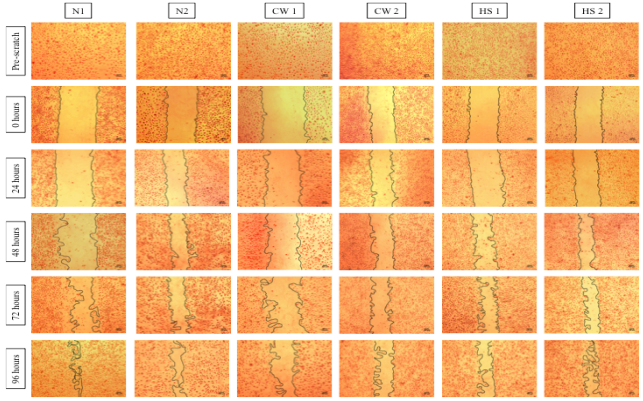


Figure 1: Keratinocyte scratch migration assay in normal (N) chronic wound (CW) and hidradenitis suppurativa (HS) keratinocytes.

Random effects mixed model was used to examine whether the pattern of change in scratch surface area differed between diagnostic groups over time. In the model predicting wound size, error bars show 95% confidence interval (CI) for normal group. Group x time interaction was significant for chronic wound at 72 and 96 hours ($p<0.05$) and trending towards significant for HS group at 96 hours (p 0.063).

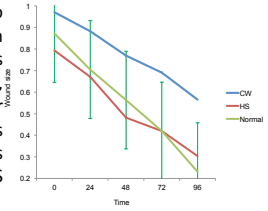
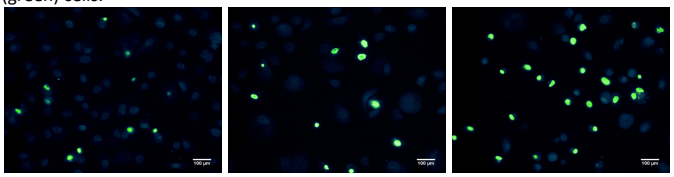


Figure 2: Change in scratch surface area over time. Error bars show 95% CI for normal group.

Viability Assay

Cell viability was assessed at 96 hours using the ratio of viable (blue) to non viable (green) cells.



Normal | Hidradenitis | Chronic wound

Figure 3: Representative fluorescence microscopy images of normal, chronic wound and hidradenitis suppurativa specimens.

The viability ratio (%) was significantly higher in normal compared to chronic wound keratinocytes (86.15 ±4.13 vs. 59.94±11.68, p= 0.0055). There were no significant differences between hidradenitis suppurativa and normal keratinocytes (86.15 ±4.13 vs. 81.16±2.24, p= 0.077).

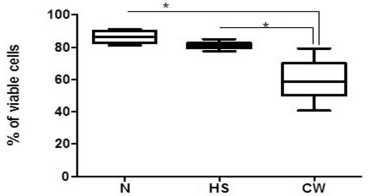


Figure 4: Cell viability ratio (%) at 96 hours for normal (N), hidradenitis (HS), and chronic wounds (CW).

Discussion

Primary cultured keratinocytes isolated from the skin of subjects with wounds and hidradenitis suppurativa show significant differences in m and viability. The keratinocyte scratch assay is a useful in vitro met studying human keratinocyte function in chronic wounds and HS.

Cell viability assay found that keratinocytes from normal skin had h viability than chronic wound keratinocytes. Defective keratinocyte viabili account for the differences in rate of scratch closure between the clinical This is clinically important since it suggests, that even when removed f chronic inflammatory milieu that contributes to delayed healing in wounds, an inherent difference in keratinocyte behavior is seen.

This study has several limitations that merit discussion. The sample size small because keratinocyte isolation and culture is time consuming and We have only been able to culture a small number of samples thus anticipate that as workflows and systems improve we will be able to valid findings in additional independent samples.

The next step for this work will be to investigate cytokine profiles on effluent at various time points to see if there are measurable differe keratinocyte cytokine production that correlate with the scratch clos viability assay findings.

Conclusion

Primary cultured keratinocytes from chronic wound hidradenitis suppurativa demonstrate differenc migration and viability compared to keratinocytes is from normal human skin. Keratinocyte dysfunctio contribute to delayed healing in chronic wounds. This merits further investigation.

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