Characterization of a Three-Dimensional (3D) Organotypic Skin Model using Keratinocytes and Mesenchymal Stem Cells Immortalized by hTERT

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ASCB Poster #: P711

Abstract

In this study, we compared primary keratinocytes (ATCC® No. PCS-200-010) to hTERT immortalized keratinocytes (Ker-CT; ATCC® No. CRL-4048™), co-cultured with either primary fibroblasts (ATCC® No. PCS-201-010), primary adipose-derived mesenchymal stem cells (MSCs; ATCC® No. PCS-500-011), hTERT-immortalized fibroblasts (BJ-5ta; ATCC® No. CRL-4001™), or hTERT-immortalized MSCs (hTERT-MSCs; ATCC® No. SCRC-4000™). We confirmed that both primary keratinocytes and Ker-CT are able to fully differentiate into skin equivalents in a 3D culture model when co-cultured with primary fibroblasts, primary MSCs, BJ-5ta, or hTERT-MSCs. To confirm the functionality of the co-culture models, both the primary keratinocytes and the Ker-CT air-liquid interface (ALI) co-cultures were subjected to a scratch assay. Re-epithelialization occurred in both cell lines, and interleukin 8 (IL-8) showed an increase in expression from day 0 to day 1 and 3, corresponding to migration of cells into the wound. The continuous nature of the Ker-CT cell line makes it an invaluable model for the research of keratinocyte biology, as it eliminates the issue of short life span and donor variation seen with primary cells.

Introduction

- Keratinocytes undergo a program of terminal differentiation that results in a potent barrier against microbial infection, water loss, and chemical attack.
- Primary keratinocyte cultures are important cell models for the study of normal and pathological biology of the cutaneous epithelia.
- Keratinocyte differentiation is promoted by factors secreted by cells in the dermis.
- Primary keratinocytes can form skin equivalents that mimic the architectural features and behavior of normal skin in a 3D organotypic culture model in an ALI.
- Primary keratinocytes have finite lifespan in culture, which greatly restricts their use as an *in-vitro* cellular model.
- The constitutive overexpression of the catalytic subunit of telomerase, hTERT, allows for extended passaging of cells with the benefit of little loss of normal cellular physiology.
- hTERT-immortalized keratinocytes cultured with primary fibroblasts, primary MSCs, BJ-5ta, or hTERT-immortalized MSCs comprise a 3D model of the epidermis with high value for investigating drug delivery, toxicity of cosmetics, and the pathology of psoriasis.

Results

hTERT-immortalized and primary stroma-secreting cells directly and indirectly promote primary keratinocytes to form a stratified, 3D, differentiated epidermis

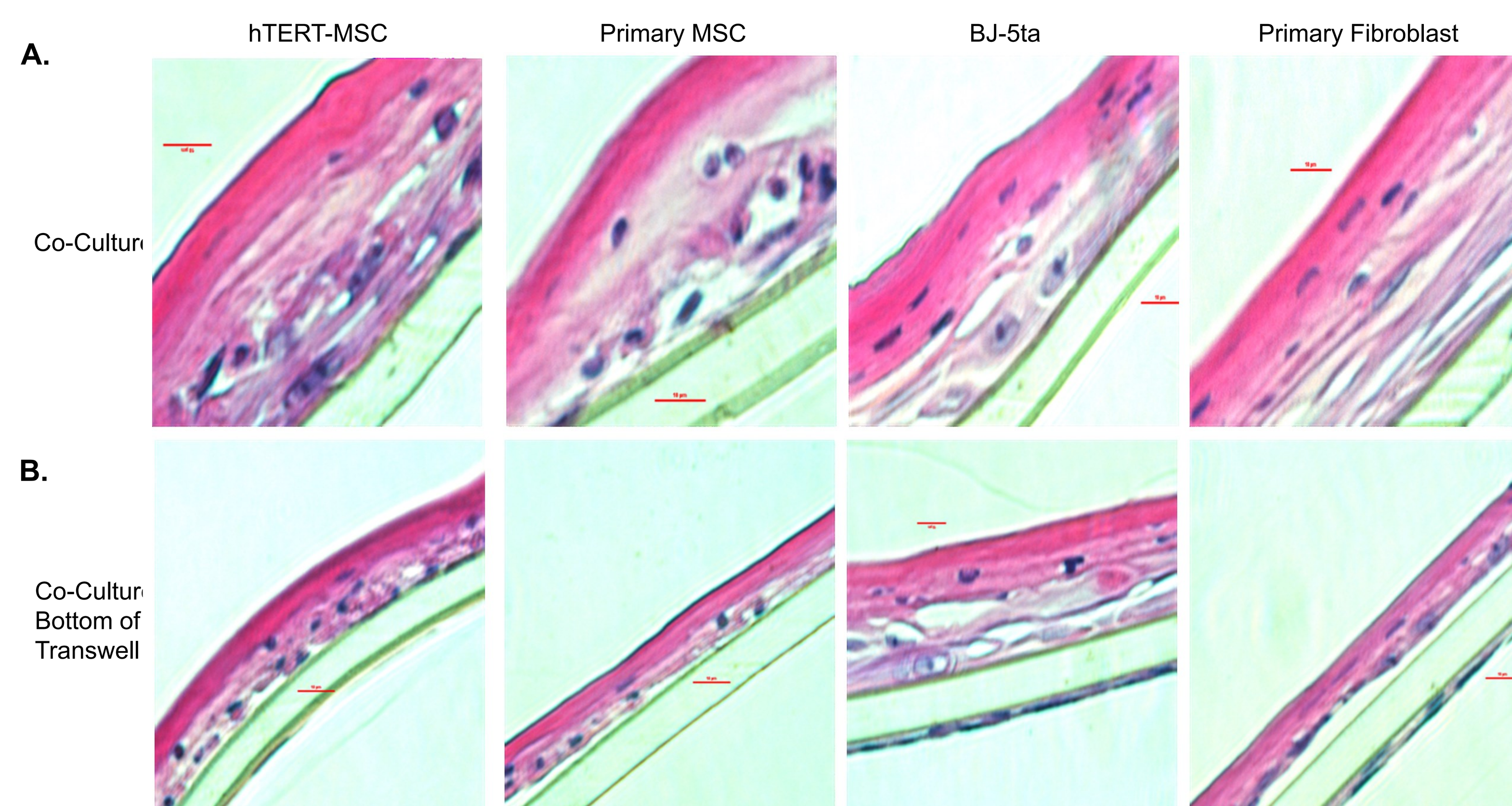

Figure 1. Hemotoxylin and Eosin (HE)-stained cross sections of primary keratinocytes co-cultured with hTERT-immortalized and primary MSCs or fibroblasts differentiated for 21 days. Primary fibroblasts, MSCs, or their hTERT-immortalized counterparts were seeded onto A) the Transwell™ (Corning) apical chamber or on B) the underside of the Transwell. The fibroblasts and MSCs were allowed to adhere for 24 hours in Mesenchymal Stem Cell Basal Medium (ATCC® PCS-500-030) supplemented with Mesenchymal Stem Cell Growth Kit (ATCC® PCS-500-040). BJ-5ta and primary dermal fibroblasts were grown in Eagle's Minimal Essential Medium (ATCC® 30-2003) supplemented with 10% Fetal Bovine Serum (ATCC® 30-2020). The keratinocytes were then seeded into the apical chamber and allowed to adhere for 4 hours. Cultures were then maintained according to Kalabis *et al.*: Media was changed to epidermalization medium 1 (EPM1). After 3-5 days media was removed and EPM2 was added to the basal chamber only, with media changes every 2-3 days[1]. The cells remained in culture for 21 days, were cross-sectioned, and then subjected to HE staining. All images were taken at 20 x magnification.

hTERT-immortalized and primary stroma-secreting cells directly and indirectly promote Ker-CT to form a stratified, 3D, differentiated epidermis

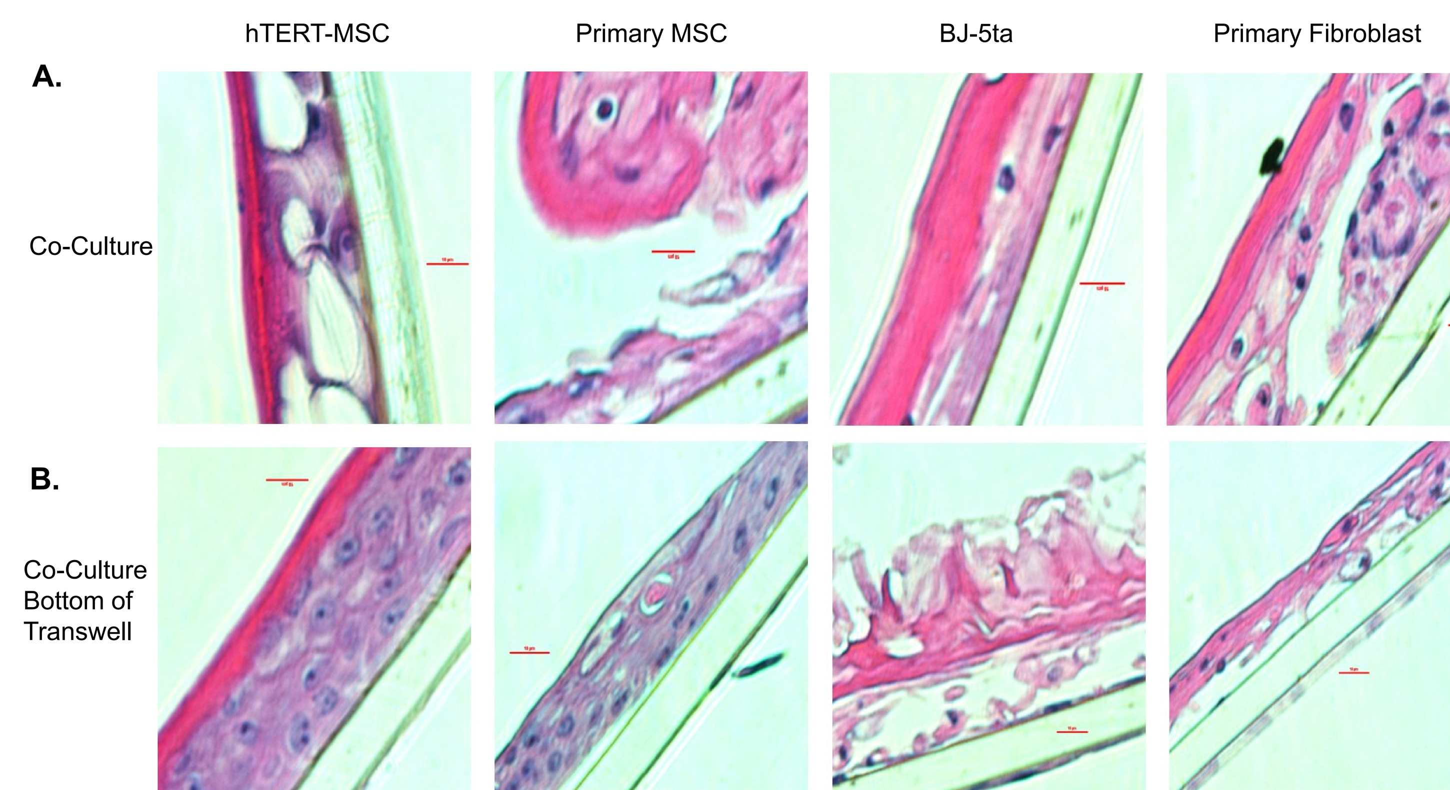

Figure 2. HE-stained cross sections of Ker-CT co-cultured with hTERT and primary MSCs or fibroblasts, then differentiated for 21 days. Primary fibroblasts, MSCs, or their h-TERT-immortalized counterparts were seeded onto A) the Transwell apical chamber or B) the underside of the Transwell, then co-cultured with Ker-CT and processed as in Figure 1.

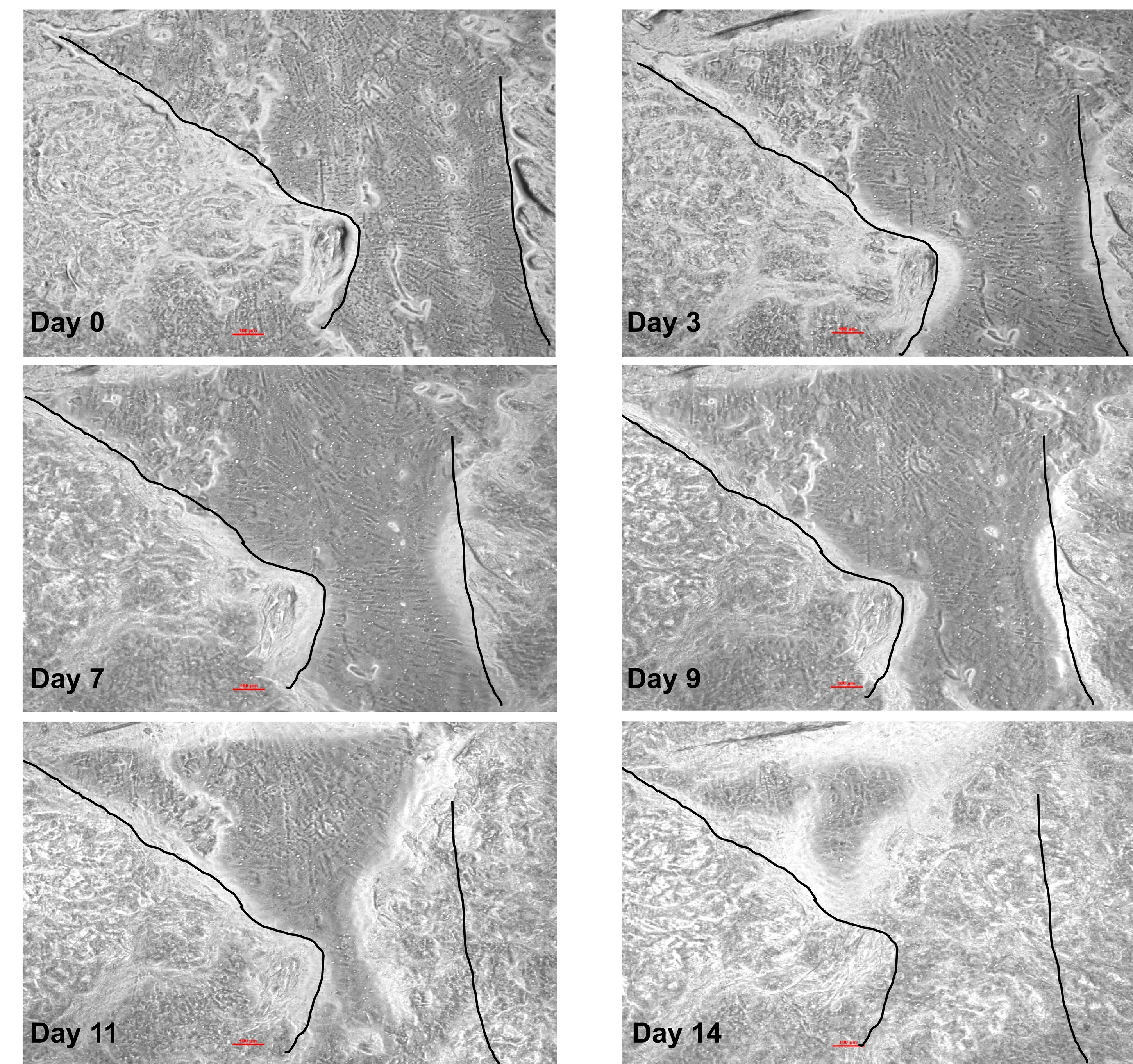

Figure 3. Ker-CT co-cultured with hTERT-MSC healed after scratch test. Ker-CT cells were seeded onto Transwells containing hTERT-MSCs in the basal chamber as described in figure 1. After 21 days of differentiation, the apical chamber of the Transwells were scratched using a P1000 tip. Media (EPM2) was changed every 2-3 days for a total of 14 days. Phase images at a 10 x magnification were taken at the indicated days to observe the wound healing. Black lines indicate the size of the wound at day 0.

Primary keratinocytes and Ker-CT provide an *in vitro* model of epidermal inflammation

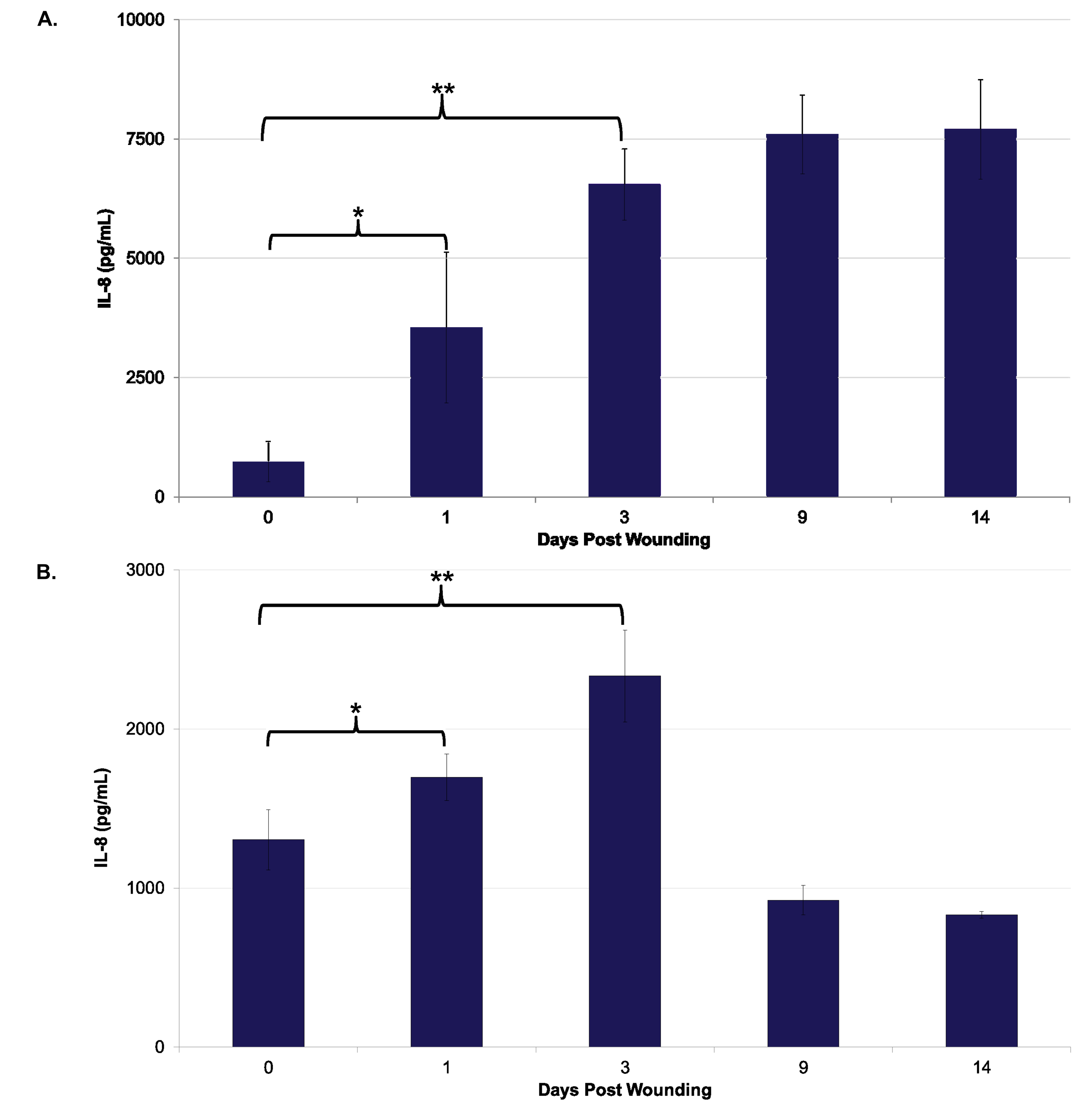

Figure 4. Concentration of IL-8 secreted into the basal media of the primary and immortalized keratinocytes during wound healing. Basal medium of organotypic A) primary keratinocytes and B) Ker-CT at day 0 (before wounding) and after wounding were collected. Concentrations were then analyzed using the Luminex™100 (Luminex) platform (n=2, performed in triplicate, *represents $p<0.005$ vs day 0, ** represents $p<0.00001$ vs day 0).

Summary

- *In vitro* epidermal assay models were established using an ALI Transwell system, primary epidermal keratinocyte cells or Ker-CT, and primary fibroblasts, MSCs, BJ-5ta, or hTERT-MSCs.
- Primary keratinocytes and Ker-CT displayed stratification and terminal differentiation.
- Ker-CT filled in wounds after being subjected to a scratch assay.
- Ker-CT displayed a similar inflammatory response as compared to the primary keratinocytes after wounding.
- Immortalized keratinocyte co-culture model provides a consistent and robust *in vitro* system which increases consistency between assays by decreasing the lot to lot variability of primary cells.

References

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