

## Keratin-based Biomaterial Significantly Attenuates Cellular Injury Responses Following UVB Exposure

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**Statement of Purpose:** Burn wound healing is a complex process that includes phases of inflammation, granulation tissue formation, re-epithelialization, and remodeling. While human skin is a complex biological milieu, several cell types have been well-documented for their role in wound healing, including keratinocytes (epidermis) and dermal fibroblasts (dermis). If the cellular healing response is hyperactivated or left unchecked, these wounds often contract and form hypertrophic scars that create both functional and cosmetic problems. One potential solution is the keratin-based biomaterial wound treatment known as KeraStat™ Burn Gel. This biomaterial has been shown in pre-clinical porcine models to speed healing and reduce burn conversion in partial thickness burns, but also to dramatically reduce inflammation in 1<sup>st</sup> degree sun or thermal burns. These data led to the hypothesis that cells important in the wound healing and remodeling process would attenuate pro-inflammatory responses when exposed to keratin proteins. To test this hypothesis, human fibroblasts and keratinocytes were injured using UVB radiation, and following treatment with keratin biomaterials, their inflammatory and matrix gene expression profiles were examined using RT-PCR arrays.

**Methods:** Human gene expression profiles were monitored in pathway-focused real-time PCR plates, RT<sup>2</sup> Profiler Arrays (SA Biosciences), each of which includes 84 pathway-specific primer sets to measure either inflammatory cytokines and chemokines or extracellular matrix components and adhesion molecules. Keratinocytes or fibroblasts were plated and exposed to a damaging dose of UVB radiation (100 mJ/cm<sup>2</sup>), followed by immediate treatment with either a vehicle control (cell culture media) or several doses of the KeraStat™ Burn Gel formulation (0.0001–1 mg/mL, w/v). RNA was extracted at day 0 (sham UVB), day 1, day 2, and day 4 post-UVB radiation with TRIzol (Invitrogen) and purified with RNeasy Plus (Qiagen). A cDNA library was prepared for each sample with the RT<sup>2</sup> first strand synthesis kit (Qiagen), and plates were prepared according to the manufacturer's instructions (*n*=4 for each condition). Data were analyzed using an RT<sup>2</sup> profiler array-specific template on the Qiagen website, and statistical significance was determined using ANOVA.

**Results:** Data from these experiments showed that UVB radiation produced significant changes in expression for several key gene clusters in both keratinocytes (representative data shown) and fibroblasts (data not shown). These changes included several members of the collagen (shown in Figure 1 top), integrin, and matrix metalloproteinase (MMP) families, as well as key pro-inflammatory chemokines and cytokines. However, when these cells were exposed to keratin biomaterials, they demonstrated significantly attenuated gene expression changes in matrix and inflammation pathways of interest to the point that they displayed expression profiles that

approximated an uninjured normal cell. These responses were not limited to a particular class or subset of genes, suggesting that keratin biomaterials provide a broad protective, stabilizing environment that promotes wound healing.

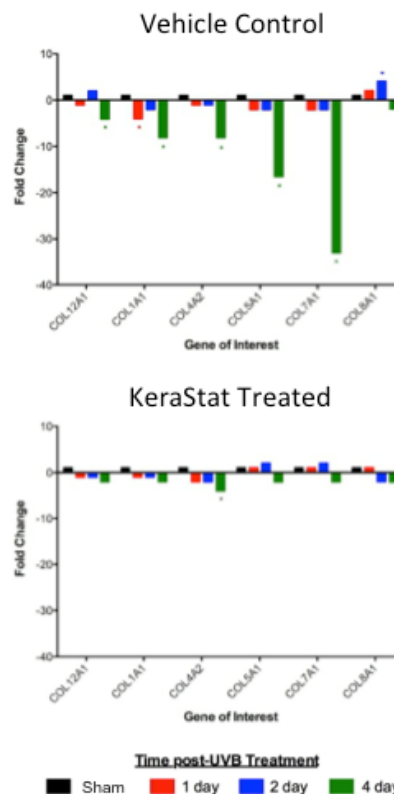


Figure 1. KeraStat™ Burn Gel significantly attenuates the cellular response to UVB damage.

**Conclusions:** We report dramatic, broad attenuation of a radiation burn injury response in two human cell lines critically involved in wound healing following treatment with KeraStat™ Burn Gel. We postulate that while the markers of inflammation and extracellular matrix components affected by this treatment are rather broad, the keratin proteins in this biomaterial, serve as an indicator to the cellular environment that the cells are uninjured, thereby acting in a protective capacity by limiting the harmful effects of inflammation. Further studies in animal models of wound healing and hypertrophic scarring are pending to evaluate the efficacy of KeraStat™ Burn Gel on thermal and radiation injuries.

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