

## EXPRESSION OF AN HBD-3 TRANSGENE IN KERATINOCYTES USING AN *EX-VIVO*, NON-VIRAL STRATEGY PRODUCES AN ANTIMICROBIAL HUMAN SKIN SUBSTITUTE

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Infection remains a significant obstacle in cutaneous wound treatment despite current medical advances. The innate immune system modulates the expression of host defense peptides (HDPs) to combat infection throughout the course of wound healing. Enhancement of antimicrobial properties inherently present in keratinocytes is a potentially useful therapeutic strategy. Towards this goal, the novel NIKS human keratinocytes were stably transfected *ex vivo* with the human beta defensin-3 (hBD-3) transgene to produce an antimicrobial three-dimensional biologic dressing.

The NIKS human keratinocyte cell line was stably transfected with a full length human  $\beta$  defensin-3 transgene by electroporation. Expression of the transgene was verified using both RT-PCR and qPCR for hBD-3 mRNA. Monolayer culture of NIKS<sup>hBD-3</sup> contained approximately 800-fold more hBD-3 mRNA than unmodified NIKS. Three-dimensional skin tissue generated by organotypic culture of NIKS expressing hBD-3 (NIK<sup>hBD-3</sup>) possessed barrier function and the histologic features of interfollicular epidermis. Furthermore, NIK<sup>hBD-3</sup> skin tissue contained approximately 100 ng hBD-3/mg total protein. Notably, no cytotoxic effects of hBD-3 expression were observed. An *in vitro* antimicrobial assay was used to quantify antimicrobial activity of conditioned media from NIK<sup>hBD-3</sup> tissue compared to unmodified NIKS tissue. hBD-3 was readily detected in conditioned medium from NIK<sup>hBD-3</sup> tissue and caused a 50% reduction in the growth of *Staphylococcus aureus mprF* (*S. aureus mprF*). Moreover, in an *in vivo* murine burn model, a 72-hour application of NIK<sup>hBD-3</sup> skin substitute tissue on an infected wound bed resulted in a 1 log (90%) reduction in bacterial growth.

These results demonstrate that sustained delivery of hBD-3 by a bioengineered skin tissue with tissue-specific biological function results in a therapeutically relevant reduction in growth of the pathogenic bacterial strain, *S. aureus mprF*, in a model of infected cutaneous wounds.