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Correspondence and requests for materials should be addressed to R.K. (Roger.Kaspar@TransDermInc.com)

* Current address: Department of Developmental and Cell Biology, University of California at Irvine, Irvine, CA

** Current address: Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea

Visualization of plasmid delivery to keratinocytes in mouse and human epidermis

Emilio González-González[1,2], Yeu-Chun Kim[5,6**], Tycho J. Speaker[7], Robyn P. Hickerson[7], Ryan Spitler[1,2*], James C. Birchall[8], Maria Fernanda Lara[1,2], Rong-hua Hu[9], Yanhua Liang[9], Nancy Kirkiles-Smith[10], Mark R. Prausnitz[5], Leonard M. Milstone[9], Christopher H. Contag[1,2,3,4] & Roger L. Kaspar[2,7]

[1]Molecular Imaging Program at Stanford (MIPS), Stanford University School of Medicine, CA, USA, [2]Department of Pediatrics, Stanford University School of Medicine, CA, USA, [3]Department of Radiology, Stanford University School of Medicine, CA, USA, [4]Department of Microbiology & Immunology, Stanford University School of Medicine, CA, USA, [5]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [6]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, [7]TransDerm Inc., Santa Cruz, CA, USA, [8]Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3NB, UK, [9]Department of Dermatology Yale University School of Medicine, New Haven, CT, USA, [10]Department of Immunology, Yale University School of Medicine, New Haven, CT, USA.

The accessibility of skin makes it an ideal target organ for nucleic acid-based therapeutics; however, effective patient-friendly delivery remains a major obstacle to clinical utility. A variety of limited and inefficient methods of delivering nucleic acids to keratinocytes have been demonstrated; further advances will require well-characterized reagents, rapid noninvasive assays of delivery, and well-developed skin model systems. Using intravital fluorescence and bioluminescence imaging and a standard set of reporter plasmids we demonstrate transfection of cells in mouse and human xenograft skin using intradermal injection and two microneedle array delivery systems. Reporter gene expression could be detected in individual keratinocytes, in real-time, in both mouse skin as well as human skin xenografts. These studies revealed that non-invasive intravital imaging can be used as a guide for developing gene delivery tools, establishing a benchmark for comparative testing of nucleic acid skin delivery technologies.

The impressive progress in identifying the underlying causes of many epidermal disorders, including genodermatoses, have led to potential therapeutic targets and promising intervention strategies; however, translation of such novel therapies into the clinic has not been achieved.[1] Nucleic acid-based therapies are strong candidates for many skin disorders that lack effective treatment options.[2–4] However, realization of this goal will require new methods for efficient delivery through the stratum corneum and efficient uptake and utilization of the nucleic acid therapeutic by targeted cells. At present, no nucleic acid skin delivery methodologies exist that result in safe and efficient delivery across these biological barriers without causing pain or tissue damage. To address this need, a consortium of researchers with diverse yet complementary expertise in skin delivery was established[5] to address the following specific aims: 1. develop and test patient-friendly skin delivery technologies that effectively and efficiently deliver nucleic acids to skin; 2. combine appropriate and promising technologies to overcome the delivery barriers and; 3. enable direct comparison of existing delivery technologies through developing, and making available to the research community, standardized and validated tools, including intravital imaging systems, mouse and human xenograft skin models and common reagents. Validated reagents and verified skin models that allow meaningful comparisons are required for effective development of nucleic acid delivery systems.

Successful development of nucleic acid therapies would clearly benefit from non-invasive imaging modalities that enable visualization of expression patterns in skin strata in real time. The use of a dual reporter system (e.g., expression of luciferase and fluorescent protein from a single plasmid construct) allows for increased data acquisition since both bioluminescence and fluorescence imaging modalities can be utilized. *In vivo* bioluminescence imaging (BLI) has been shown to be a versatile and sensitive technique for tracking and quantifying gene expression and assessing delivery efficiency using reporter genes such as firefly luciferase.[6–8] Despite its strengths, BLI has relatively low resolution and therefore detection and identification of individual reporter-positive cells