Establishing cytotoxicity screening assays in human epidermal keratinocytes and dermal fibroblasts

Emma Hoffman[1], Morgan Cook[1], Scott Kelsey[1], Kevin Grady[2] and Anshika Sharma[1]

1: Cell Biology Center of Excellence, Lonza, Durham, NC, USA. 2: Lonza, Walkersville, MD, USA.

The skin is the first barrier in the human body, protecting against damage caused by UV light and chemical substances as well as protecting internal structures against microorganisms and mechanical trauma. It is composed of three layers: the epidermis, containing keratinocytes as the most frequent cell type, the dermis, which consists mainly of connective tissue and fibroblasts, and the hypodermis, the deepest layer which is made up of adipose and connective tissue. Following damage, injury, or during the normal turnover of cells, the appropriate proliferation response of both fibroblasts and keratinocytes is essential for the regeneration and repair process to occur effectively. Delays in this process can lead to chronic wounds and an increased potential for bacterial colonization, while excessive and uncontrolled proliferation can lead to benign or malignant neoplasms. Despite the common clinical use of various antimicrobial agents to prevent delays in skin healing and the standard use of chemotherapy agents to treat skin cancers, these compounds can have adverse effects on healthy skin cell viability. Evaluation of these cytotoxic effects during the drug discovery process is crucial to understanding how to achieve a balance between adequate clinically-relevant outcomes and minimizing toxicity or off-target effects that could

damage skin or prevent wound healing. Here, we present a workflow for cytotoxicity assays targeting normal human epidermal keratinocytes (NHEKs) and normal human dermal fibroblasts (NHDFs). We validated this workflow by exposing both cell types to common antimicrobial or chemotherapy agents over acute and chronic timescales.

Methods

Lonza NHEK and NHDF cells (n = 3 donors for each cell type) were plated at 6500-8000 cells/cm^2 and cultured in KGM® Gold (Lonza) or FGM® 2 medium (Lonza), respectively, in standard T-75 flasks. Cells were cultured for 6 days, with a media change on day 2 or 4, until reaching confluency. Cells were then passaged and seeded into four 96-well plates each for NHEKs and NHDFs at a density of 3200 – 4800 cells/cm^2 per well. The four plates per cell type were comprised of two drug treatments (NHEKs exposed to eight concentrations of doxorubicin ranging from 0.1 – 2 µM or eight concentrations of chlorohexidine ranging from 0.25 – 10 µM; NHDFs exposed to eight concentrations of camptothecin ranging from 0.001 – 2 µM or exposed to eight concentrations of chloroxylenol ranging from 25 – 500 µM) by two exposure times (acute vs chronic). Plates were returned to the incubator at 37°C and 5% CO_2. Plates designated for acute exposure were cultured with media changes every 2 – 3 days until reaching 40 – 60% confluency, at which point they were exposed to their respective cytotoxic compound and cultured for 24 hours. Plates designated for chronic exposure were cultured with media changes every 2 – 3 days until reaching 10 – 20% confluency, at which point they were exposed to their respective cytotoxic compound and cultured for 120 hours, with a media change (including the cytotoxic compound) on day 2 or 3. Chronic toxicity experiments were started at a lower confluency to prevent the cells from becoming over-confluent by 120 hours, which would impact cell health, potentially confounding effects from the cytotoxin and also making it difficult to baseline against the negative control treatment. Cytotoxicity was measured using the Lonza ViaLight® Plus Cytotoxicity Bioassay Kit to quantify total cellular ATP relative to untreated control cells as a proxy for viability. A slightly modified protocol was used for this study, whereby the cell lysis reagent was added to the sample wells as normal, but the ATP monitoring reagent (AMR) was added to the white 96-well assay plate. Aliquots of cell lysis were transferred from the sample plate to the new plate containing the AMR, after which the ViaLight® Assay was run per manufacturer instructions.

Results and discussion

For both cell types in this study, we utilized cytotoxins representing two different types of compounds. Doxorubicin (NHEKs) and camptothecin (NHDFs) are commonly used chemotherapy agents, while chlorohexidine (NHEKs) and chloroxylenol (NHDFs) are commonly used anti-microbial compounds employed in wound healing protocols and as antiseptics.

As seen in other studies[1], exposure time plays a significant role in overall toxicity, with short exposure times maintaining higher viability than a prolonged dosing at the same concentration (Figure 1). Across both cell types and all compounds tested, IC_{50} values at 120 hours were significantly lower than at 24 hours, with lethal effects observed at the highest concentrations during chronic exposure. Conversely, short, acute exposures did not follow the same trend, with only chlorohexidine (Figure 1C) exhibiting lethality at the highest concentration tested. There was also a great deal more donor-to-donor variation in cytotoxic responses at 24 hours compared to 120 hours, which suggests that variation in individual donors' sensitivities to a particular compound were overwhelmed with consistent exposure over time. Similarly, donors with higher IC_{50} values at 120 hours did not always exhibit a relatively high IC_{50} value, suggesting that individual responses to specific cytotoxic compounds are driven by interactions between donor health, genetics, and mechanisms of action of the compound. Despite these nuances, the trajectory of dose-response curves was near-identical across donors for every compound and exposure time period. Overall, our experimental results validate this protocol as a robust, high-throughput workflow for determining the cytotoxic effects of various compounds on human dermal fibroblasts or keratinocytes.

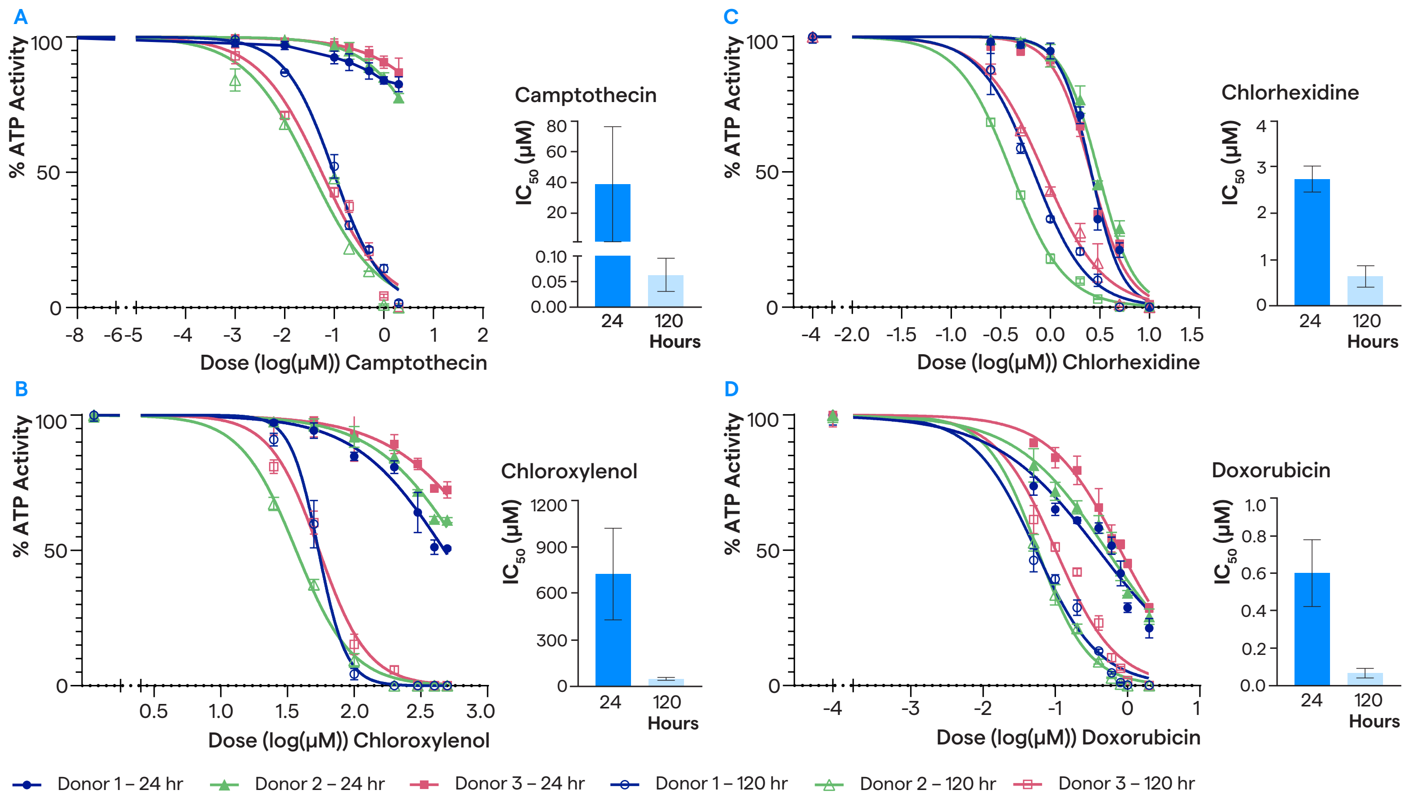

Figure 1.
Dose-response curves and IC_{50} values for Normal Human Dermal Fibroblasts (NHDFs) exposed to (A) camptothecin or (B) chloroxylenol, and Normal Human Epidermal Keratinocytes (NHEKs) exposed to (C) chlorhexidine or (D) doxorubicin for 24 hours and for 120 hours. In dose-response figures, each color represents the same donor for the 24 hour (closed symbol) and 120 hour (open symbol) timepoints to clearly represent the responses of each individual donor across two different exposure periods. In the bar graphs, each bar represents the mean of responses of the three donors. Error bars represent ±1 SD from the mean.

References:

1. **Babich** *et al*. (1995). An *in vitro* study on the cytotoxicity of chlorhexidine digluconate to human gingival cells. *Cell Biology and Toxicology* 11: 79-88.

Contact us

North America
Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +49 221 99199 400
scientific.support.eu@lonza.com

International
Contact your local Lonza distributor
Customer Service: +1 301 898 7025
scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

Learn more.

All trademarks belong to Lonza Group Ltd. and its affiliates (collectively and individually, "Lonza"), and are registered in the USA, EU and/or CH, or used in common law, or belong to third-party owners and are used for only informational purposes. All third-party copyrights have been reproduced with permission from their owners. User assumes all risks of product use and handling. Lonza makes efforts to include accurate and up-to-date information. However, Lonza makes no representations or warranties, express or implied, including as to the accuracy or completeness of information, or the use or handling of these products. The user is responsible for determining if the products supplied by Lonza and the information and recommendations given by Lonza are (i) suitable for intended process or purpose, (ii) are in compliance with environmental, health and safety regulations, and other regulations in the regions and countries where they are purchased, offered for sale, marketed, sold, and used and (iii) will not infringe any third party's intellectual property rights. The user bears the sole responsibility for determining the existence of any such third-party rights, as well as obtaining any necessary licenses and approvals. For more information, including regarding legal disclaimers, Lonza's intellectual property rights, and how Lonza collects, uses and protects personal information: www.lonza.com/legal, https://www.lonza.com/about-us/strategy/intellectual-property and www.lonza.com/privacy

©2026 Lonza. All right reserved.

CD-SP115 03/26

bioscience.lonza.com