

EVALUATION OF MTT METABOLISM AS A MEANINGFUL INDICATOR OF VIABILITY IN HUMAN CORNEAL EPITHELIAL TISSUE MODELS. MD Hines, SD Gettings and BC Jones. Avon Products, Inc. Suffern, NY USA.

Cellular viability is routinely measured by the uptake and reduction of tetrazolium salt, MTT, to an insoluble formazan dye by cellular microsomal enzymes. With the emerging utilization of tissue equivalent models, the MTT bioassay has often been used for measuring cellular viability, however it has recently been reported that MTT only measures the viability in the basal layer of cells while cells in the suprabasal layers go undetected. To evaluate the ability of the MTT assay to measure the cell viability in tissue equivalent models, we examined the cellular viability of two commercially available human corneal models ([MatTek EpiOcular OCL-200](#) and SkinEthic HCE) with multiple viability markers. First, we determined that the amount of formazan formed by healthy, non-treated tissue constructs increased linearly between 0.5 and 3 hr. of exposure to MTT. Histological analysis of cryopreserved tissue models incubated with MTT revealed that all nucleated cell layers in both tissue models reduced MTT demonstrating metabolically viable cells in the appropriate cell layers. An opposite staining pattern was visualized in tissue models treated with propidium iodide, a dye that penetrates nonviable cells, with only cells in the upper most (granular) layer retaining the dye. Further, rhodamine 123, which localizes to the mitochondria of viable cells, produced a similar distribution pattern to MTT in both tissue models. When the tissue constructs were treated with Triton X-100 to induce cellular damage, we measured expected decreases in MTT reduction after 10, 30 and 60 min exposures. Visual assessment of histological sections after Triton X-100 supports these quantified values with an approximate amount of cells retaining the formazan crystals. Our experiments demonstrate that the traditional MTT method exhibited conversion linearly with time and by multiple cell layers in the tissue constructs. In addition, results from the MTT method were comparable to other cytotoxicity dyes. Thus, we conclude that our data support the use of MTT as a cell viability marker as a stand-alone method.