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Cytometric Viability of Human Corneal Epithelial Cells Grown on a Biomaterial

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INTRODUCTION: *In vitro* tissue culture studies were used to compare the viability and possible inflammatory response of human corneal epithelial cells, SV 40 transformed (HCE-T), grown on tissue culture plastic, (TCP), and a polymeric hydrogel biomaterial (BioM).

METHODS: Cell viability was by flow cytometry using Molecular Probes, Live/Dead –Cell Viability kit (LDCV). HCE-T cells were grown in UltraCulture™ media w/o antibiotics (1.5 X 10^5cells/ml), removed from tissue culture flasks and seeded to 24 well flat bottomed plates and to the biomaterial surface. Supernatants were collected and frozen for cytokines evaluation. HCE-T cells were trypsinized, rinsed, spun and characterized by double staining with calcein (Ca) and ethidium homodimer -1 (EthD). The LDCV endpoint was flow cytometric analysis carried out at 488 nm on a BDFACSCailbur™ 4 color unit cytometer. Subpopulations were identified and sorted by regions for live (green fluorescent) and dead (red fluorescent) cells. Dead cell controls were treated with BAC (50ppm) for 15 minutes before analysis. Representative HCE-T cells populations from TCP and BioM were counted. Cytokine stimulation was by PMA at 100ng/ml. Cytokine analysis was done using the BD CBA assay for human pro-inflammatory cytokines.

RESULTS: Cell morphology was examined by phase microscopy. Cell populations were 5x 10^4cells/ml-BioM and 8 x 10^4cells/ml-TCP (Fig 1). BioM flow cytometry were 95% live cells (LR) and 4% membranes (UR) after 4 days growth. TCP cells were 98% (LR) and 2% (UR). Only BAC treated cells exhibited 98%(UL) for dead cells (Fig 2). Cytokine levels were normalized for cell counts, supernatant volume and TCP final pg/ml concentrations were then used for normalization. TCP and BioM PMA HCE-T cells were stimulated for TNF-a, IL-8 & IL-6 (Table 1).

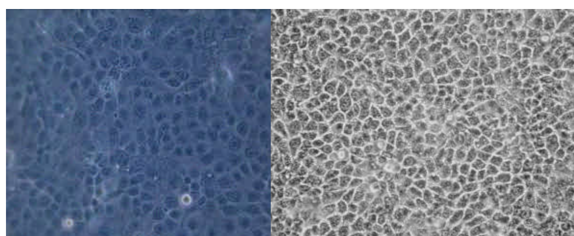

Fig. 1: HCE-T morphology after 4 days growth: (left) HCE-T monolayer grown on BioM. (right) HCE-T monolayer grown on TCP.

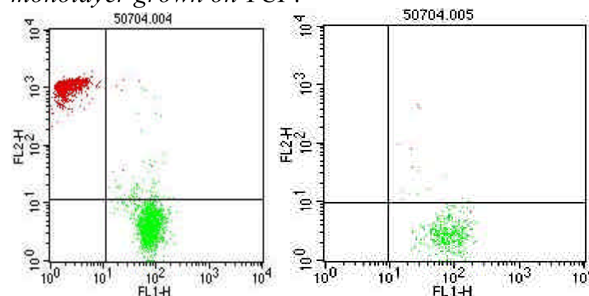

Fig. 2: Flow cytometry of HCE-T stained with LDCV: (left) HCE-T mixed live- dead (BAC) treated population grown on TCP. (right) HCE-T grown on BioM with gated live/dead regions.

surface	IL 1ß	TNF a	IL10	IL8	IL12 p70	IL6
TCP	1	1	1	1	1	1
TCP/ PMA	1	26	4.5	13	1	4
BioM	0.8	0.8	1.7	1.2	0.5	1.3
BioM/PMA	0.5	14	0.5	6	0.9	2

Table 1. Cytokine ratios of HCE-T normalized to TCP. Signf. at a ratio >2.0

DISCUSSION & CONCLUSIONS: HCE-T cells are viable BioM as compared to TCP. BioM is a viable surface for HCE-T cell growth and attachment. Short term exposure to BAC killed HCE-T cells. Surface material differences may attribute to the approximate 38% growth inhibition of BioM to compared TCP. HCE-T cytokines can be stimulated on both surfaces with PMA. BioM did not up-regulate pro-inflammatory cytokines compared to TCP HCE-T cells

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