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EpiOral & EpiGingival | Data Sheet

Features

- Buccal and Gingival Phenotypes
- 3D, Highly Differentiated
- Normal Human Cells
- Serum-Free Medium, Highly Reproducible
- *In Vivo*-Like Lipid Profile
- Highly Reproducible
- Easily Handled Cell Culture Inserts
- Quantifiable, Objective Endpoints
- Ideal for Irritation, Oral Pathology and Basic Studies

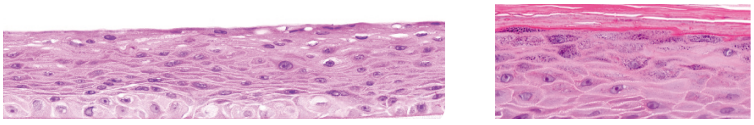
EpiOral & EpiGingival

To enable *in vitro* study of irritation, oral pathologies, and basic oral cavity phenomena, Mattek has developed the EpiOral series of tissue models. Mattek's EpiOral and EpiGingival tissues consist of normal, human-derived epithelial cells. The cells have been cultured to form multilayered, highly differentiated models of the human buccal (EpiOral) and gingival (EpiGingival) phenotypes. The tissues, which are cultured on specially prepared cell culture inserts using serum free medium, attain levels of differentiation on the cutting edge of *in vitro* cell culture technology. The EpiOral and EpiGingival tissue models exhibit *in vivo*-like morphological and growth characteristics which are uniform and highly reproducible. EpiOral is a multilayered tissue consisting of an organized basal layer and multiple non-cornified layers analogous to native human buccal tissue (Figure 1).

Both tissues express cytokeratin K13 in the all layers except the most apical ones and weakly express cytokeratin K14 in the upper layers of the tissue (Figure 3). The tissues also produce the naturally occurring antimicrobial peptides called human beta defensins (HBD). The ORL-200 constitutively expresses HBD-1 and HBD-3 but not HBD-2 (Figure 4). The GIN-100 tissue weakly expresses HBD-3 in all layers except the stratum corneum, expresses HBD-1 weakly only in the apical layers, and does not express HBD-2 (Figure 5). Lipid analysis of the tissues revealed the presence of only Ceramide 2 (Ceramide NS) in EpiOral and Ceramides 1, 2, and 3 (also referred to as Ceramides EOS, NS, and EOHP/NP, respectively) in the EpiGingival tissue, overlapping normal lipid profiles of *in vivo* tissue.

Various industrial and toxicology laboratories are actively seeking alternatives to expensive clinical or whole animal testing. Oral care, personal care, and pharmaceutical companies have initiated *in vitro* toxicology testing to evaluate their raw materials and final product formulations. Companies utilize antibiotic/antifungal free EpiOral tissue (ORL-200-AFAB) to grow various commensal and pathogenic bacteria in order to study their effects on the oral tissues. Figure 6 shows the effects of SLS, a common active ingredient of dentifrices, on the viability of ORL-200 tissue. As shown therein, a linear relationship exists between tissue viability and the SLS concentration. Such an assay can be used to predict oral irritancy of oral care products. A growing body of data indicates that EpiOral and EpiGingival effectively provide an inexpensive, non-animal means to assess oral irritation, toxicology, and pathology related issues.

Histology of EpiOral & EpiGingival



EpiOral EpiGingival

Figure 1. EpiOral (non-cornified), buccal phenotype. The EpiGingival tissue is also multilayered except that the apical layers are cornified, similar to *in vivo* gingival tissue (Figure 2).

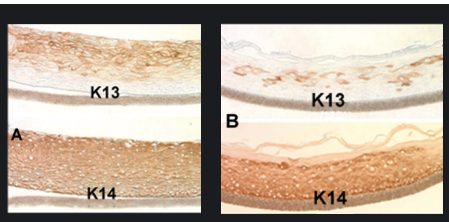


Figure 3. Immuno-staining of: A) EpiOral and B) EpiGingival tissue for cytokeratin K13 and K14. Negative control (no antibody) tissue did not stain, similar to basal layer in EpiOral K13 section in A).

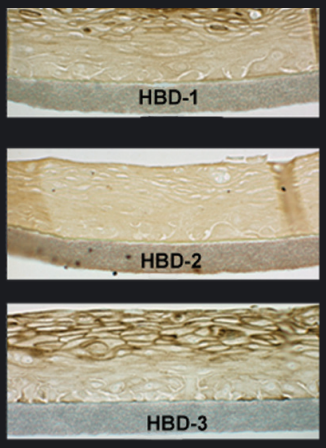


Figure 4. Immuno-staining of the ORL-200 tissue for: human beta defensins (HBD). The negative control side without the primary antibody is identical to HBD-2 slide.

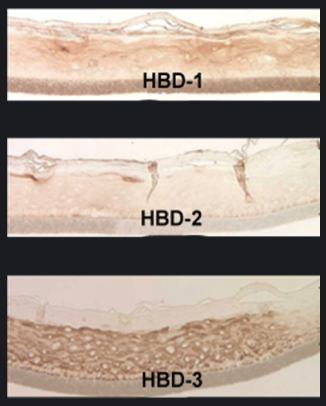


Figure 5. Immuno-staining of the GIN-100 tissue for: A) human beta defensins (HBD). The negative control slide without the primary antibody is identical to HBD-2 slide.

The protocols for using EpiOral and EpiGingival are clear and straightforward. The tissues have been utilized with several common tests of cytotoxicity and irritancy, including MTT and IL-lα. mRNA can easily be harvested to analyze gene expression and the culture medium can be analyzed using ELISA assays to measure cytokine release. Technicians find the rigid substrate design of EpiGingival easy to handle and manipulate in routine repetitive testing environments and scientists find that they are able to perform discriminating tests due to low background interference.

Oral Tissue: Dose Response Effect of SDS (40 µL Dose)

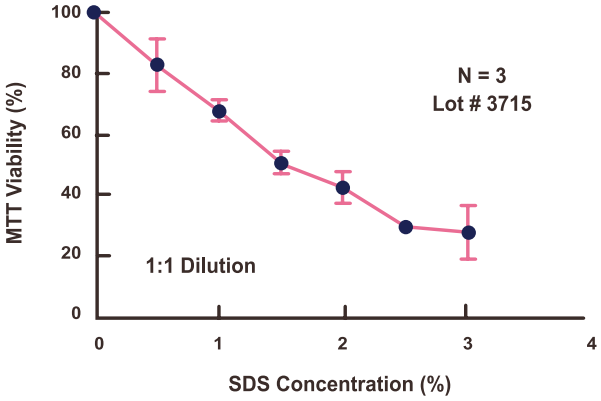


Figure 6. Effect of SDS solutions (40 mL) on ORL-200 tissue viability following exposure for 1 hour. SDS concentrations were chosen to be in range normally present in toothpastes (0.0 - 3.0%).

Lipid Species	Buccal (n=14)	Std. Dev	ORL-200	Gingival (n=14)	Std. Dev	Gin-100
PL	76.3	8.9	93.1	78.7	6.7	86.4
GSL	23.0	7.8	3.7	16.9	6.0	3.9
AGC	0.0	0.0	0.0	0.0	0.0	0.0
CER AH	0.0	0.0	0.00	0.79	0.33	0.00
CER AP	0.0	0.0	0.00	0.0	0.0	0.00
CER NH	0.0	0.0	0.00	0.88	0.43	0.00
CER AS	0.0	0.0	0.00	0.0	0.0	0.00
EOHP / NP	0.0	0.0	0.00	0.47	0.16	0.78
CER NS	0.72	0.32	2.4	1.7	0.52	5.8
CER EOS	0.0	0.0	0.00	0.58	0.18	0.71

Key: PL=Phospholipid, GSL=Glucosphingolipid, AGC=Acyl glucosylated ceramide, CER AH=ceramide 7, CER AP=ceramide 6, CER NH=ceramide 5, CER AS=ceramide 4, CER EOHP/NP=ceramide 3, CER NS=ceramide 2, CER EOS=ceramide 1 (1) 1Ponec M, Weerheim A, Lankhorst P, Wertz PW: New acylceramide in native and reconstructed epidermis. Journal of Investigative Dermatology 120:581-588, 2003.

MATTEK
Now Part of Sartorius

Mattek North America Headquarters
200 Homer Ave
Ashland, MA 01721
US: + 1 (508) 881-6771

For further information, visit
www.mattek.com or email
support@mattek.com