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In Vitro Model of the Epithelial Barrier

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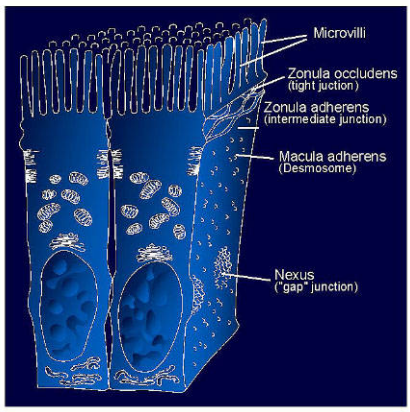
Introduction

Epithelial cells fulfill important functions in different parts of the human body. They form borders which limit and protect the organism, compartmentalize the extracellular environment and serve as transport pathways. Oral stratified epithelium forms the boundary between the oral cavity and the subepithelial tissue. Important functions of the oral epithelium are the protection and prevention of the body from microbial invasion. Especially in the gingival sulcus area, the alterations of the epithelial layer and subgingival extension of a biofilm may lead to periodontal destruction and loss of teeth.Individual cells in epithelial sheets are interconnected by a set of specialized intercellular junctions. Tight junctions (TJ) are the most apical of these intercellular structures and form a border between the apical and the basolateral cell surface domains (Farquhar and Palade, 1963) It is possible to maintain the polarity of epithelial cells in culture which also has been reported for MDCK cell line (Gonzales Mariscal et al., 1985). These cells are able to form polarized monolayers thereby expressing tight junctions and behave as a simple epithelium. Its function is characterized by a high transepithelial electrical resistance (TER) which can be measured in vivo. This property corresponds to the morphology of tight junctions (Meyle et al., 1999). The aim of this study was to compare the ability of cell lines and primary human gingival keratinocytes to develop an in vitro epithelium and to study the characteristics of this system.

Model Of Intercellular Connections Between Epithelial Cells



Schematic illustration of possible intercellular connections of epithelial cells.

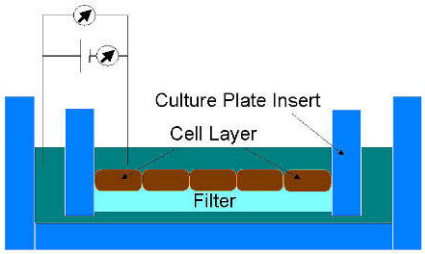
Material and Methods

Isolation and growth of human gingival keratinocytes
Biopsies of the buccal gingiva from the distal region of the upper jaw were taken after local anaesthesia using a disposable biopsy punch (Stiefel Laboratorium, Offenbach, Germany) with a diameter of 5 mm from 15 healthy volunteers. After extensive washing with PBS-/- the tissues were digested with Dispase II (2.4U/ml; Roche Molecular Biochemicals, Mannheim, Germany) for 2 hours at 37°C. The epithelial layer was removed from the underlying connective tissue and dissolved with 0.05% Trypsin-EDTA (Gibco-BRL, Karlsruhe, Germany) for 10 min in order to obtain a single cell suspension. The cells were seeded into 25 cm2 cell culture flaks (Corning Costar, Bodenheim, Germany) in serum-free keratinocyte growth medium (BioWhittaker, St. Katharinen, Germany).

Cultivation of cell lines
The high resistance strain of MDCK cells (MDCK I; kindly provided by Prof. Dr. K. Simons, Heidelberg, Germany) grew in MEM, supplemented with 10% FCS, 2mM glutamine and antibiotic/antimycotic solution (Gibco-BRL, Karlsruhe, Germany). In experiments using all-trans retinoic acid (Sigma-Aldrich, Steinheim, Germany), hormone depleted FCS was used.

Experimental protocol and measurement of electrical resistance
The different cell types wer seeded on Transwell-Col (R) filter inserts (4x105 cells/insert) in a 6-well plate (Corning Costar, Bodenheim, Germany). The transepithelial electrical resistance (TER) was measured with a Millicell-ERS-System (Millipore, Eschborn, Germany). TER-measurements were repeated every day. Each insert was measured at 3 different sites and the mean values were calculated. A control without cells was also performed. The culture medium was changed daily. In experiments with all-trans retinoic acid, the medium was changed daily and retinoic acid was applied every day. Confluence was controlled daily by light microscopy.

Method Of Measuring The Transepithelial Electrical Resistance (TER)



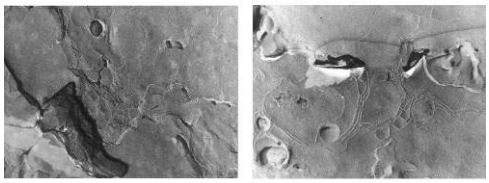
Epithelial cells are grown on permeable Collagen-coated filter inserts (0.4µm). The electrical resistance (Ohm) is determined with a volt-ohmmeter. The TER is calculated after substraction of the control (culture plate insert without cell layer) and by multiplication with the area of the insert (Ohmxcm2).

Freeze fracturing and Transmission Electron Microscopy (TEM)
The samples were fixed with 2% glutaraldehyd buffered in PBS (pH 7.4) and processed for freeze fracturing and TEM. This was done in the laboratories of Prof. Dr. H. Wolburg (University of Tuebingen, Germany).

Results

Cultured gingival keratinocytes showed largely extended tight junctions which were nearly completely associated with the p-face but not very complex.

Human Gingival Keratinocytes Are Able To Form Tight Junctions

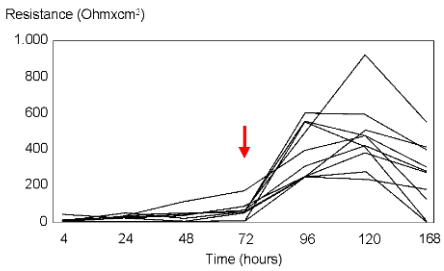


MDCK I Cells | Human Gingival Keratinocytes

Freeze fracture replica of MDCK I (high resistance, 4 days in culture) and human gingival keratinocytes (induced, 5 days in culture).Tight junctions form thick strands, are discontinous and preferentially associated with the p-face.

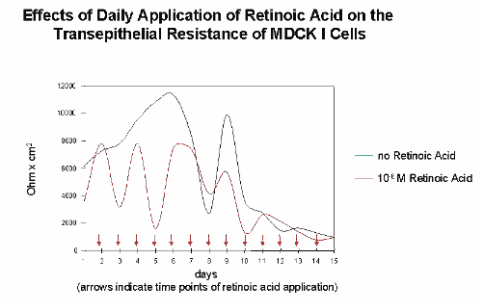
The transepithelial electrical resistance (TER) of human gingival keratinocytes is inducible after induction of differentiation. The TER values increase up to 1000 Ohmxcm2. They never reached values similiar to MDCK I (high resistance) cell layers but clearly exceeded those of control cultures.

Keratinocytes On Cellagen And The Development Of TER After Induction



Transepithelial electrical resistance of human gingival keratinocytes on filter inserts. The arrow indicates the time of medium change starting the differentiation process. Cells from 10 different volunteers were investigated.

The MDCK I (high resistance) cell line is able to produce very high TER, which increase up to 10000 Ohmxcm2. A daily application of 10-6M all-trans retinoic acid leads to an oscillation of the TER, whereas a one-fold application of 10-6M all-trans retinoic acid delays the development of a TER.



A daily application of retinoic acid leads to an oscillation of the TER of MDCK I cells. Cells were grown on filter inserts. After the measuring of the TER with a volt-ohmmeter, the culture medium was changed and 10-6M all-trans retinoic acid was added.

Discussion and Conclusions

The cultured gingival keratinocytes develop an inducible transepithelial electrical resistance. The cytokeratin pattern of the in vitro induced epithelium is comparable to the patterns found in situ (Meyle et al., 1999). Therefore this system can serve as a model system for the barrier function of the human gingival epithelium. The influences of all-trans retinoic acid and other substances on the barrier function of this in vitro system are under current investigation.
The transepithelial electrical resistance (TER) of the high resistance MDCK I cells is influenced by all-trans retinoic acid. Wanner et al. (1999) showed for epidermal keratinocytes (HaCaT cells) that desmosomes are reduced under retinoic acid treatment. Gorodeski et al. (1997) showed that retinoids regulate tight junctions in endocervical epithelial cells (CaSki cells). Therefore it is intruiging to look at the effects of retinoids on the tight junctions and desmosomes of cultured human gingival Keratinocytes and MDCK cells. This subject is being studied in our laboratory.

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- Gonzales Mariscal et al., J. Membr. Biol. 86: 113-125, 1985
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- Meyle et al., J. Periodont. Res. 34: 214-222, 1999
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This Poster was submitted on 14.05.01 by Jörg Michel.

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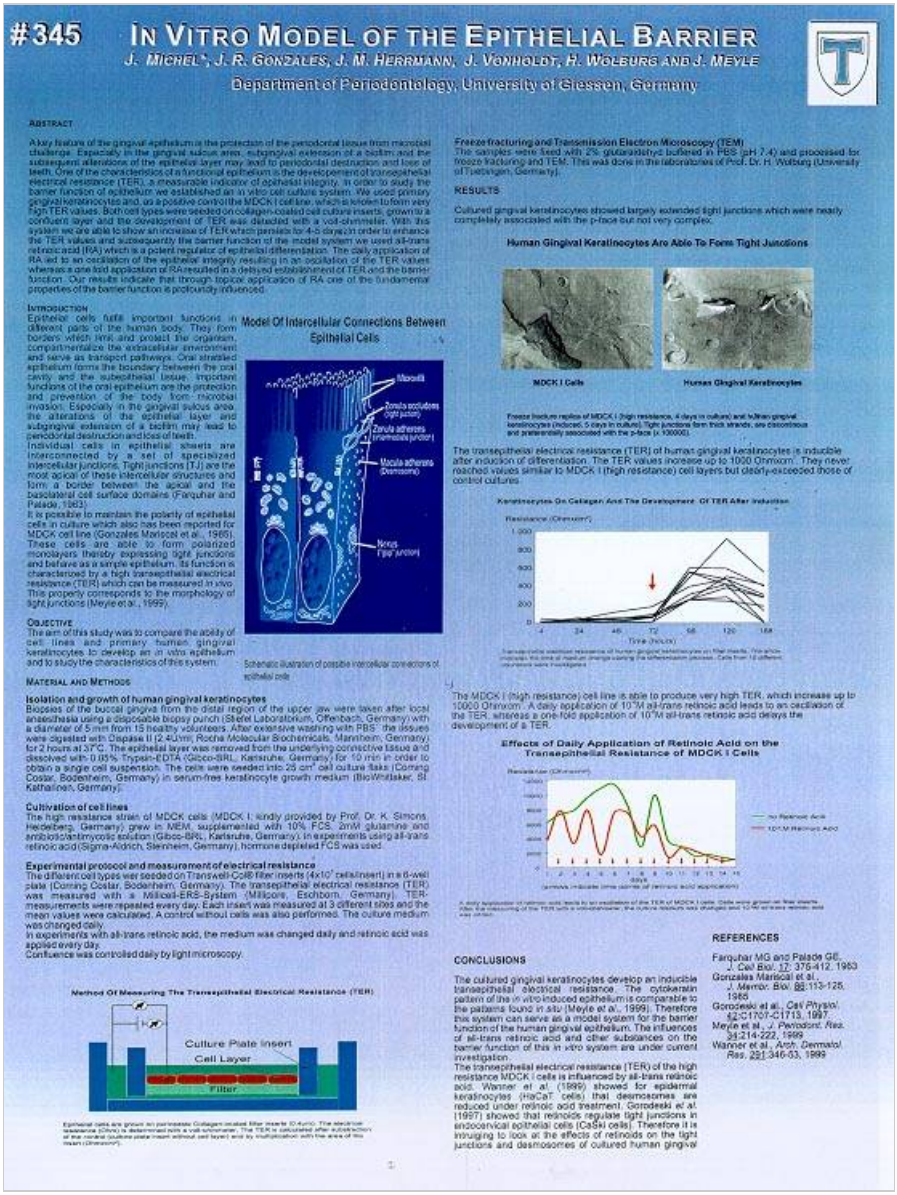

#345 In Vitro Model of the Epithelial Barrier

J. Michel*, J. R. Gonzales, J. M. Herrmann, J. Vonholdt, H. Wolburg and J. Meyle
Department of Periodontology, University of Giessen, Germany

Abstract

A key feature of the gingival epithelium is the protection of the periodontal tissue from microbial challenge. Especially in the gingival sulcus area, subgingival extension of a biofilm and the subsequent alteration of the epithelial layer may lead to periodontal destruction and loss of teeth. One of the characteristics of a functional epithelium is the development of transepithelial electrical resistance (TER), a measurable indicator of epithelial integrity. In order to study the barrier function of epithelium we established an in vitro cell culture system. We used primary gingival keratinocytes and, as a positive control the MDCK I cell line, which is known to form very high TER values. Both cell types were seeded on collagen-coated cell culture inserts, grown to a confluent layer and the development of TER was detected with a volt-ohmmeter. With this system we are able to show an increase of TER which persists for 4-5 days in order to enhance the TER values and subsequently the barrier function of the model system we used all-trans retinoic acid (RA) which is a potent regulator of epithelial differentiation. The daily application of RA led to an oscillation of the epithelial integrity resulting in an oscillation of the TER values whereas a one fold application of RA resulted in a delayed establishment of TER and the barrier function. Our results indicate that through topical application of RA one of the fundamental properties of the barrier function is profoundly influenced.

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Effects of Daily Application of Retinoic Acid on the Transepithelial Resistance of MDCK I Cells

A daily application of retinoic acid leads to an oscillation of the TER of MDCK I cells. Cells were grown on filter inserts. After the measuring of the TER cells a volt-ohmmeter, the culture medium was changed and 10^{-6}M all-trans retinoic acid was applied.

Conclusions

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References

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