



Metabolic Changes of Cells Caused by Oral Galvanism

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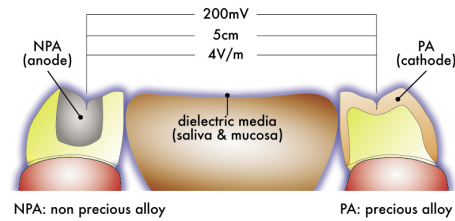


Introduction

Electrostatic voltages up to 800 mV (equivalent to 16 V/m) can be measured in vivo between different dental alloys used for the restoration of the dental arch. Caused by the differences within the galvanic series, these alloys can show signs of corrosion or produce physiologic effects through electric fields acting on the mucosal tissues.

Patients concerned complain of BMS, glossodynia or altered taste sensations. Increased field strengths between the different elements may also cause acute and chronic diseases of the oral mucosa, whereby the development of oral cancer is discussed.

This study was designed to assess physiologic reactions and changes of cells of the oral mucosa in vitro with the help of a newly developed pseudo-realistic method.



Material and Method

Material: Human oral mucosa cancer cell line UM-SSC-14C, cultivated in 5% CO₂/humidified air at 37°C with DMEM supplemented with 10% FCS, 2 mM glutamine, 100 μM β-mercaptoethanol, 2 mM nonessential amino acids stock solution, 100 U/ml penicillin, 100 μg/ml streptomycin; monolayers dissociated enzymatically (0.2% trypsin/0.05% EDTA) and plated on cell culture Petri dishes.

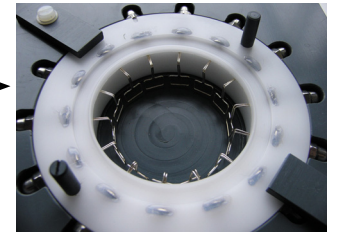
Testing device: Cell cultivation chamber with 14 electrodes connected clockwise in opposite pairs (5 cm) to the electric current (each 0.9 s) in order to prevent from polarisation of the cells and the nutrient solution.

Method: Subconfluent cell cultures exposed to voltages of 0-16 V/m (0-800 mV) for 24 hrs in steps of 2 V/m.

Agents & Antibodies: Cytokeratin-14, anti-Ki-76, anti-Cu/Zn SOD, anti-Hsp70, cleaved Caspase-3, rabbit PARP-1, gp91-phox, Nox-4, p22-phox, p47-phox, p67-phox, Cy2-GaM, Cy5-SaG, Cy3-GaR.

Fluorescence dyes & microscopy: 5-CMF-DA, H₂DCFDA, Sytox green and DiOC₂ to demonstrate metabolic changes; confocal laser-scanning-microscopy (Zeiss LSM 410/Plan with Apofluar).

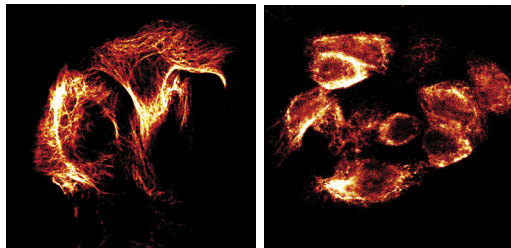
Microscopic and statistical analyses: Semiquantitative image analysis (LSM), Student t-test.



Results

Shape of cells and intracellular transport processes

The shape of the cells and direct intracellular transport processes demonstrated by Cytokeratin-14 yields impaired structural organization of the structures after electric field treatment.

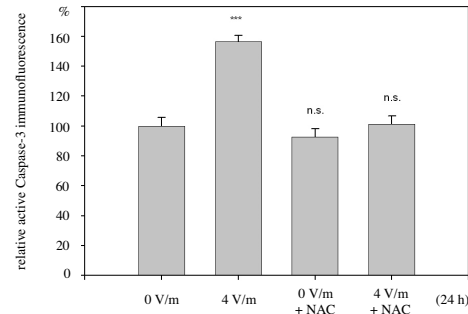


0 V/m (24 h)

4 V/m (24 h)

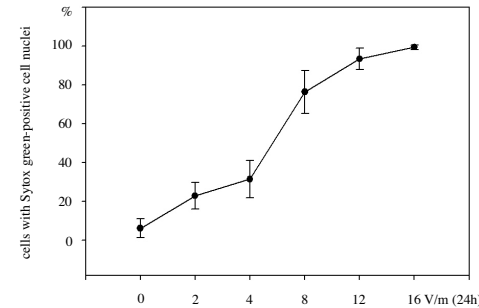
Induction of apoptosis

Electric field treatment increases cleaved Caspase-3 indicating induction of apoptosis and DNA repair. The effect is significantly (p<.001) suppressed in the presence of the anti-oxidant N-Acetyl-Cystein (NAC).



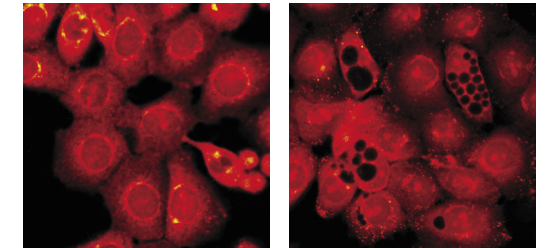
Quantity of apoptotic cells

Apoptosis clearly depends on the magnitude of the electric field as shown by evaluation of Sytox green-positive cell nuclei of cells with compromised membranes.



Formation of vacuoles

Fluorescence microscopy after DiOC₂ staining clearly demonstrates the formation of vacuoles and pyknotic cells after electric field treatment indicating induction of apoptosis.

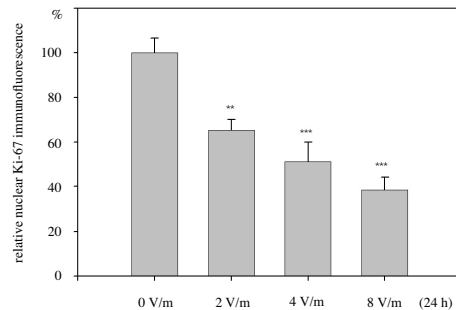


0 V/m (24 h)

4 V/m (24 h)

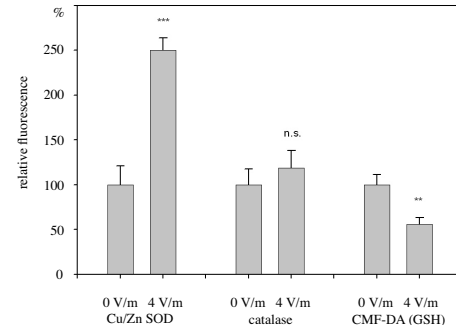
Cell proliferation

Ki-67 is a nuclear antigen that is expressed during cell proliferation. Along with electric field treatment, Ki-67 expression is impaired, indicating decreased cell cycle activity.



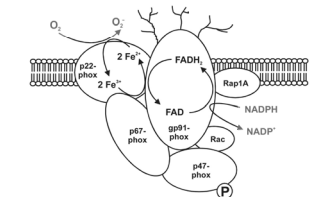
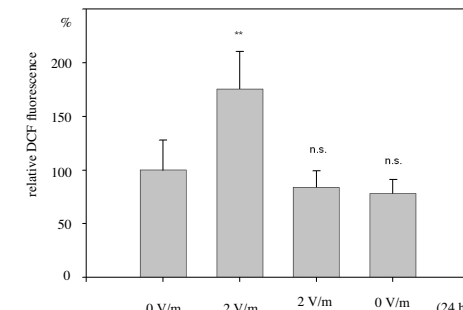
Intracellular antioxidativ defense

Electric field treatment with 4 V/m for 24 h results in a significant (p<.001) increase of Cu/Zn superoxide dismutase (SOD) and decrease (p<.01) in Glutathion (GSH) levels. However, Catalase is nearly unchanged.



Reactive oxygen species

Electric field treatment significantly increases DCF fluorescence that is indicative for increased generation of reactive oxygen species (ROS) as determined by the use of redox indicator H₂DCF-DA. However, ROS generation is suppressed in the presence of the NADPH-oxidase inhibitor diphenylen iodonium (DPI, 10 μM) and therefore, the reduction of O₂ to O₂⁻ cannot be catalyzed sufficiently by the NADPH-oxidase multi-enzym complex.



Conclusion

Electrostatic fields of only 4 V/m stimulate reactive oxygen intermediates that inhibit cell proliferation and act as a trigger for cell apoptosis.

Since cellular defense mechanisms are activated just to an inadequate degree, cells inside the electric field cannot be protected against irreversible harm.