

Int Poster J Dent Oral Med 2001, Vol 3 No 2, Poster 80

Influence of Serum Dental Alloy Eluates on Primary Human CD8±Lymphocyte-Migration

Language: English

Author(s): Georg Gassmann¹, Frank Entschladen², Kurt S. Zänker², Wolf-Dieter Grimm¹

¹University of Witten/Herdecke, Department of Periodontology, Germany

²University of Witten/Herdecke, Institute of Immunology, Germany

Date/Event/Venue:

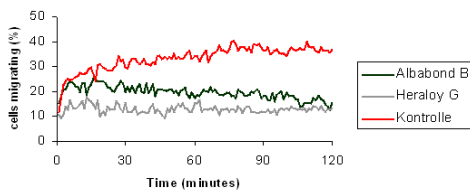
17th.- 20th September 2000

86th Meeting of the American Academy of Periodontology in Conjunction with the Japanese Society of Periodontology Honolulu, Hawaii

Introduction

Introduction and aims: Locomotion of T lymphocytes within three-dimensional collagen matrices is regulated via different signaling states of the cells. Purified human Cd8+ T cells developed a spontaneously locomoting subpopulation of about 25% of the whole population immediately after incorporation into a three-dimensional collagen matrix analyzed by time-lapse videomicroscopy. Furthermore, confocal microscopy analysis of phosphotyrosine residues, focal adhesin kinase (FAK), and protein kinase C (PKC) revealed an exclusive cellular distribution of these components, suggesting a regulation of T lymphocyte locomotion different from migration models developed for other cell types (Entschladen et al. 1997).

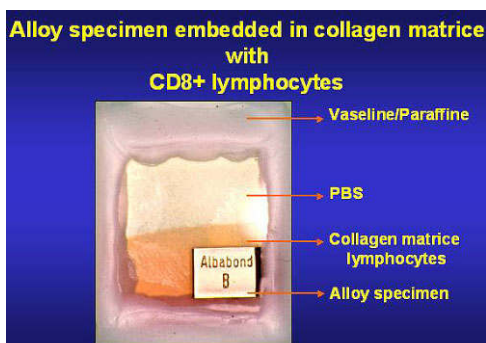
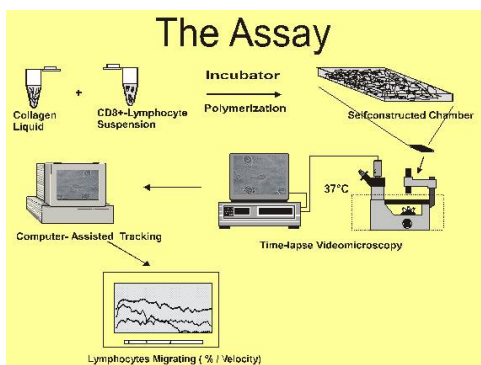
Average percentage of CD8+ lymphocyte migration on two dental alloys



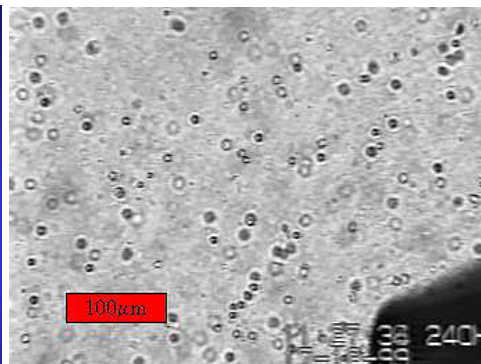
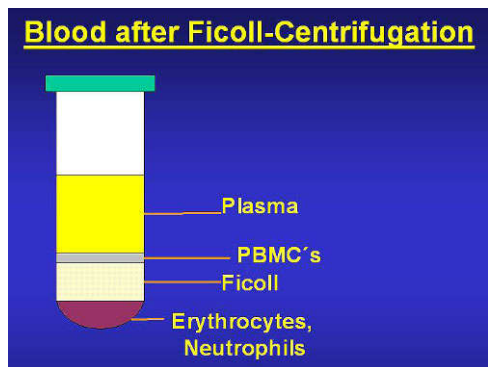
Objective

In how far is CD8+ lymphocyte Migration affected by the eluates of dental alloys?

Material and Methods



The mononuclear cell fraction was isolated from heparinized blood of healthy donors by density-gradient centrifugation using Ficoll-Hypaque. CD8+ cells were positively selected using immunomagnetic beads coated with mouse anti-human Cd8 mAb (ITI-5C2) for 10 min. at 4°. Subsequently, cell-bound beads were detached using polyclonal anti-mouse Fab Abs for 45 min. at 20°. Purified viable CD8+ cells were selected by flow cytometry. Isolated cells were maintained overnight in RPMI supplement with 2 mM L-glutamine, 10% heat-inactivated FCS, penicillin and streptomycin. No additional stimulus was added.

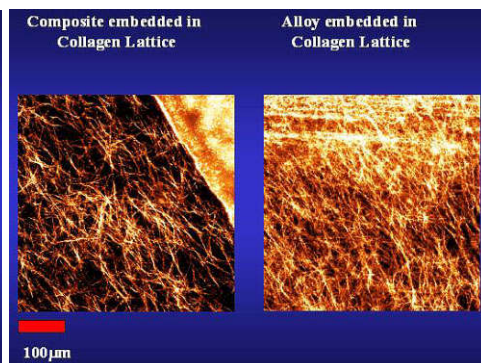
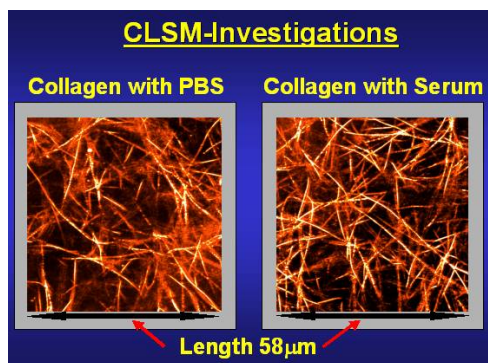


Time-lapse videomicroscopy and computer-assisted cell tracking:

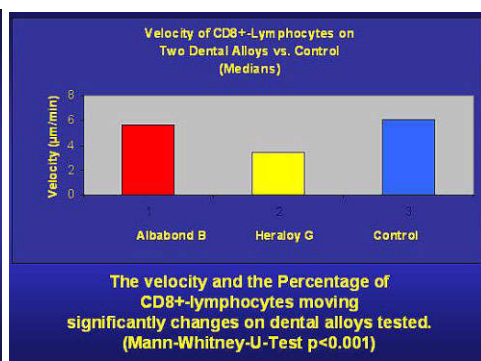
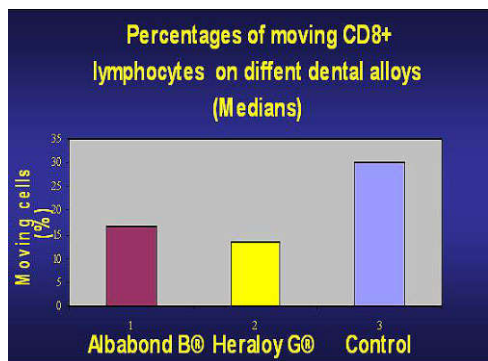
T cell locomotion within 3D collagen lattices was recorded by time-lapse video-microscopy.

Confocal Laser-Scanning Microscopy (CLSM):

Three-channel confocal microscopy was performed using an inverted confocal laser-scanning microscope (Leica TCS 4D, Bensheim, Germany). Interaction between the surrounding 3D collagen matrix and the specimens were visualized by confocal reflection in combination with the transmission image. Dual-color immunofluorescence has been performed.



Results



- Spontaneous CD8+ lymphocyte migration is reduced on the dental alloys tested.
- The reduction is different on different alloys.
- CD8+ lymphocyte migration could be a biofunctional parameter for testing biocompatibility.
- Further studies are needed to evaluate the influence of dental alloys on the signal transmitting pathways (PTK/PKC) in CD8+ lymphocytes.

Discussion and Conclusions

- Using a highly sensitive continuous time cell-tracking approach, spontaneous T cell migration in 3D collagen lattices was challenged by serum eluates of dental alloys (non precious and precious alloys).
- Reduction of cell migration was higher in precious alloy eluates than in non precious alloy eluates.
- CD8+ lymphocyte migration could be a biofunctional parameter for testing biocompatibility.
- Further studies are needed to evaluate the influence of dental alloys on the signal transducing pathways within CD8+ lymphocyte migration.

Bibliography

1. Entschladen, F., Niggemann, B., Zänker, K.S., Friedl, P. :Differential Requirement of ProteinTyrosine Kinases and Protein Kinase C in the Regulation of T Cell Locomotion in 3D Collagen Matrices.J. Immunol. 159: 3203-3210; 1997

2. Friedl, P., Entschladen, F., Conrad, C., Niggemann, B., Zänker, K.S. :CD4± lymphocytes migrating in three-dimensional collagen lattices lack focal adhesions and utilize beta 1 integrin-independent strategies for polarization, interaction with collagen fibres and locomotion. Eur. J. Immunol. 28 (8): 2331-43; 1998

This Poster was submitted on 28.05.01 by Dr. Georg Gassmann.

Correspondence address:

Dr. Georg Gassmann

Universität Witten/Herdecke
ZMK

Department of Periodontology
Alfred-Herrhausen-Straße 50
58448 Witten
Germany

Poster Faksimile:

Introduction: The cellular locomotion is a feature of diverse T cell types. Using a 3D collagen matrix migration assay in combination with confocal microscopy we investigated the locomotion behavior of CD8+ lymphocytes growing in dental material. In order to study the influence of different dental alloys, material and methods: Lymphocytes of immunologically isolated human CD8+ peripheral blood lymphocytes suspended in 3D collagen gels were recorded using time-lapse videomicroscopy. Parts of randomly selected migrating cells over a period of two hours were digitally reconstructed and quantitatively analyzed. A dental alloy free area served as a control. We evaluated two different quantitative parameters: (1) the average velocity of CD8+ cells moving and (2) the number of the migrating CD8+ cells. The percentage of CD8+ cells migrating in the presence of dental alloys was 100% compared to 100% in the control. The average migration velocity was 1.1 µm/h in the control. Comparing the velocity of the migrating lymphocytes in the control (1.1 µm/h) and in the presence of dental alloys (0.8 µm/h) we found a significant difference (p < 0.05). Discussion: It is assumed that the CD8+ cells are migrating in a 3D collagen matrix regardless of a random-walk motion induced by the components of immunologically fixed gels. (Entschladen et al. 1997) observed a spontaneous locomotion of CD8+ cells, accompanied by activated kinase phosphorylation of the focal adhesion kinase (FAK). In contrast inhibition of protein tyrosine kinase (PTK) activity was accompanied by inhibition of protein kinase C (PKC) using PP2. The PTK-inhibitor treatment is independent of tyrosine phosphorylation levels indicating that the lymphocyte locomotion is regulated by more than one signal transduction pathway. The analysis of these two "kinase-inactivated" cells can be used as an indicator for biocompatibility of dental restorations. Supported by Herdecke.

INFLUENCE OF SERUM DENTAL ALLOY ELUATES ON PRIMARY HUMAN CD8+ LYMPHOCYTE-MIGRATION
G. Gassmann¹, F. Entschladen¹, K.S. Zänker², Grimm, W.-D.^{1,2}

Departments of Periodontology, University of Witten¹ and University of North Carolina at Chapel Hill², Institut of Immunology, University of Witten¹, Germany

P# 17
universität
Witten/Herdecke

Material and Methods:

The mononuclear cell fraction was isolated from heparinized blood of healthy donors by density-gradient centrifugation using Ficoll-Hypaque. CD8+ cells were positively selected using immunomagnetic beads coated with mouse anti-human CD8 mAb (1H10C10) for 10 min at 4°C. Subsequently, cell bound beads were detached using polyclonal anti-mouse IgG (Abc) for 45 min at 30°C. Sorted CD8+ cells were selected by flow cytometry, isolated cells were maintained overnight in RPMI supplement with 2 mM L-glutamine, 10% heat-inactivated FCS, penicillin and streptomycin. No additional stimulus was added.

Time-lapse videomicroscopy and computer-assisted cell tracking: T cell locomotion within 3D collagen lattices was recorded by time-lapse videomicroscopy. Confocal Laser-Scanning Microscopy (CLSM): Three-channel confocal microscopy was performed using an inverted confocal laser-scanning microscope (Leica TCS 4D, Bannockburn, Germany). Interaction between the surrounding 3D collagen matrix and the specimens were visualized by confocal reflection in combination with the brightfield image. Dual-color immunofluorescence has been performed.

Results:

Alloy specimen imbedded in collagen matrix with CD8+ lymphocytes

Discussion:

- Using a highly sensitive continuous time cell-tracking approach, spontaneous T cell migration in 3D collagen lattices was challenged by serum eluates of dental alloys (non precious and precious alloys).
- Spontaneous cell migration was higher in precious alloy eluates than in non precious alloy eluates.
- CD8+ lymphocyte migration could be a bi-functional parameter for testing biocompatibility.
- Further studies are needed to evaluate the influence of dental alloys on the signal transducing pathways within CD8+ lymphocyte migration.