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Influence of Serum Ti-Dental-Monomaterial-Eluates in Comparison to Precious-Dental-Alloy-Eluates on Primary Human CD4$^+$-Lymphocyte-Migration.

Language: English

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Abstract

Introduction:
Active cellular locomotion is a feature of diverse T cell types. Using a 3-D collagen matrix migration model in combination with computer-assisted cell tracking for reconstruction of migration paths and confocal microscopy, we investigated the locomotion behavior of CD4$^+$ lymphocytes governing cell-dental material interactions due to the influence of different dental alloys. We tested the hypothesis of whether changes of lomotory behavior after induction of chemotaxis could be ascribed to changes of the regulatory signal transduction of migration. We did this on the basis of the trading that spontaneous locomotion of T lymphocytes was regulated by PTK activity and was distinct from a second, protein kinase C (PKC)-dependent type of migration inducible by PKC-activating phorbol ester due to different serum eluates of dental alloys.

Material and methods:
Human peripheral CD4$^+$ cells were isolated from heparinized blood of healthy donors by density-gradient, centrifugation using Ficoll-Hypaque. CD4$^+$ cells were positively selected using immunomagnetic beads coated with mouse anti-human CD4$^+$mAb. Subsequently, cell-bound beads were detached using polyclonal anti-mouse Fab antibodies. More than 98% of the cells were viable, as assessed by propidium iodide staining and flow cytometry. For collagen lattices preparations 2,5 x 10^5 cells were mixed with 100µl of buffered collagen solution (pH 7,4) containing 1,67 mg/ml collagen type I in minimal essential Eagle's medium. Locomotion of CD4$^+$ lymphocytes suspended in type I collagen gels was recorded using time-lapse video-microscopy. Paths of 30 randomly selected locomoting cells over a period of two hours were digitized, reconstructed and quantitatively analysed. A dental alloy free assay served as a control. We evaluated two different quantitative parameters using Ti-dental monomaterials in comparison to precious dental alloys: (1) the average percentage of CD4$^+$ cells moving and (2) the velocity of the migrating CD4$^+$-cells due to the influence of serum dental material eluates.

Results:
We could show a reduction of average percentage of CD4$^+$ cells migration in the presence of precious alloys (25,6%±5,8 for "high-precious-alloys", 53,1%±5,6 for "reduced-precious-alloys", 28%±5,8 for palladium-based alloys) in comparison to the non-reduction of the average migration in the Ti-group. Concerning the velocity the same deminishing tendency could be seen for precious and palladium-based alloys (range from 1,65µm/min±2,0 up to 3,0µm/min±2,4) in comparison to the highly biocompatible results of Ti-monomaterials (4,8µm/min2,4).

Discussion:
We presume that the CD4$^+$ cells are migrating in a 3D collagen matrix migration model in a "random-walk" fashion influenced by the components of serum dental material eluates. Further the developed test could be used as an indicator for biocompatibility of different dental materials. Conclusion: The results of our newly developed test showed a higher biocompatibility of Ti-monomaterials in comparison to precious dental alloys.

Introduction

In previous investigations our group could show that CD8$^+$ lymphocyte migration is influenced by precious dental alloy eluates. The purpose of this study was to show whether the same diminishing effect on CD4$^+$ lymphocyte migration was to be seen due to the same alloys in comparison to titanium dental monomaterial eluates.

Material and Methods

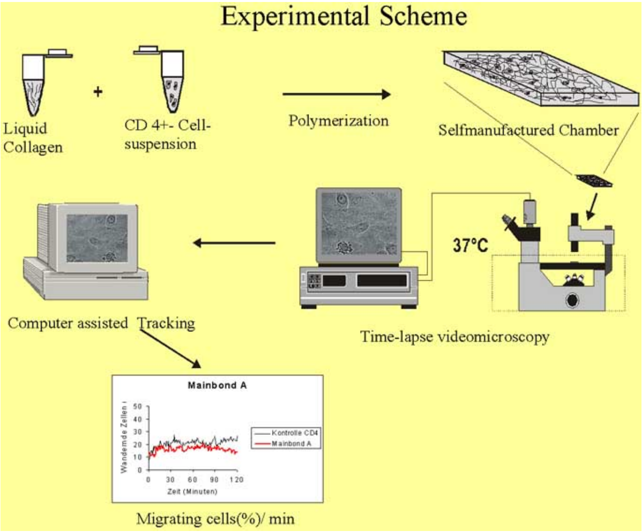

Fig.1: Liquid fetal bovine collagen was mixed with 4 X 10^5 CD4+ lymphocytes. Polymerization was initiated with Sodiumbicarbonate (7.5%).The mixture was transfered to the selfconstructed chamber containing the specimen

Fig.2: Selfconstructed chamber with one of the alloy specimen embedded in the collagen matrice.

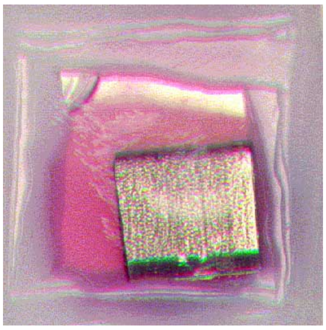

Fig.3: Selfconstructed chamber with a titanium specimen embedded in the collagen matrice.

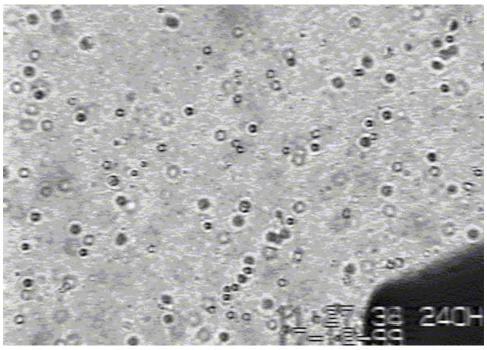

Fig.4: 30 cells in the aspect of the monitor were randomly chosen. When one of the cells left the field of the monitor anotherone was chosen. Two hours of cell migration were reduced to two minutes by time-lapse videomicroscopy. Every second in this two minutes the coordinates of the cells migrating were taken. The trials were repeated five times. So per alloy and for titanium we gained 600 data that were statistically refered to the 600 data resulting from the controls. Significance was tested applying the Mann-Whitney-U-test.

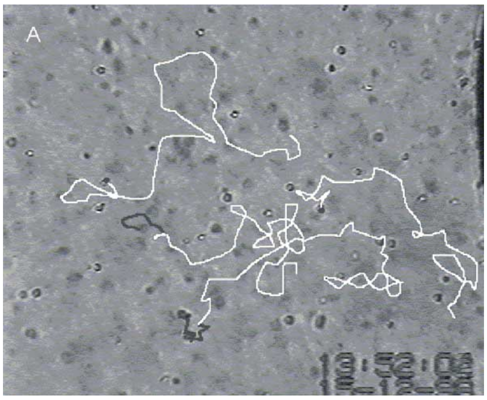

Fig.5: This figure visualizes the pathways of four CD4+ llymphocytes. It can be seen that it iis of great interest not only to determine the starting and the endpoint of migration. We analyzed the percentage and the velocity of the cells migrating on six different dental alloys (HeraloyG®,Hera GG®, Albabond B®, Mainbond A®, Bio Herador N®, Mainbond EH®) and titanium.

Results

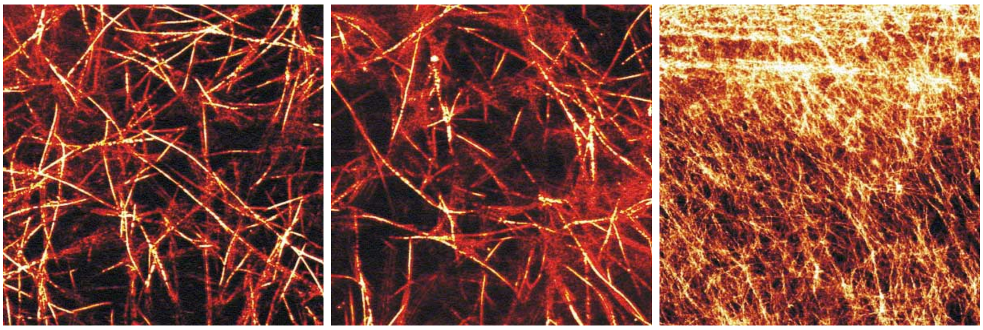

Fig.6 A

Fig.6 B

Fig.6 C

Fig.6: The referability of the results due to the dental materials was dependent on the fact that there was no influence caused by neither the serum nor the specimen brought into the model. Neither serum (A) caused differences compared with the collagen structure within PBS (B), nor did the alloy (C).

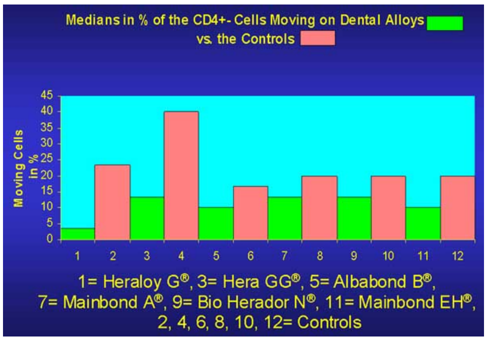

Fig.7: Different reducements of the percentage due to different alloys.

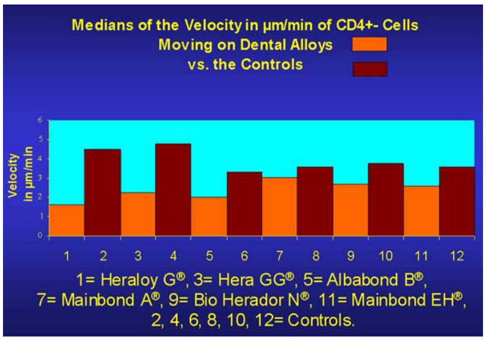

Fig.8: Reducements of the velocity parallel to the percentage.

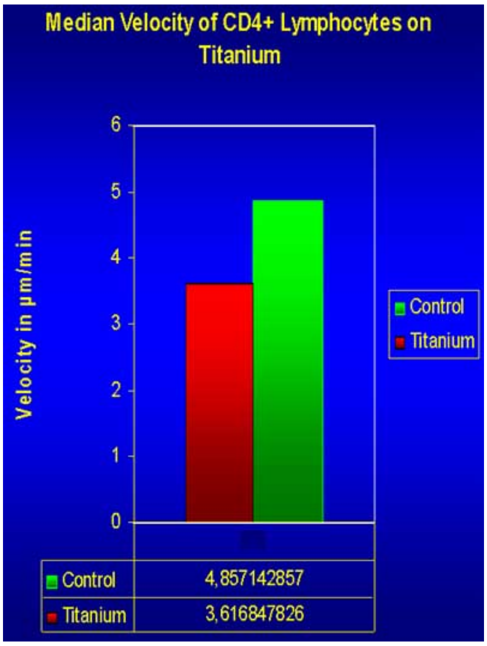

Fig.9: Titanium caused an enhencement of the CD4+ lymphocytes migrating.

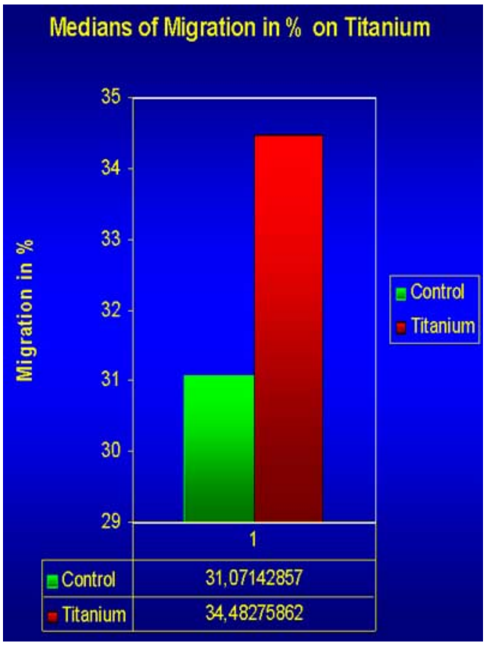

Fig.10: The median of the velocity of CD4+ cells migrating was reduced to 72%.

Conclusions

- Spontanous CD4$^+$ lymphocyte migration is reduced on all alloys tested except titanium.
- Reducement of cell migration is due to the material and reproducable.
- CD4$^+$ lymphocyte migration is a biofunctional parameter suitable for testing biocompatibility.
- Further studies are needed to evaluate the influences of the single components on cell migration.
- Future research has to show the influences on the signal transduction pathways.

This poster was submitted by OA Dr. med. dent. Georg Gassmann.

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Poster Faksimile:

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