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New in vitro-Model of Impaired Immune Response in Periodontitis

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Introduction

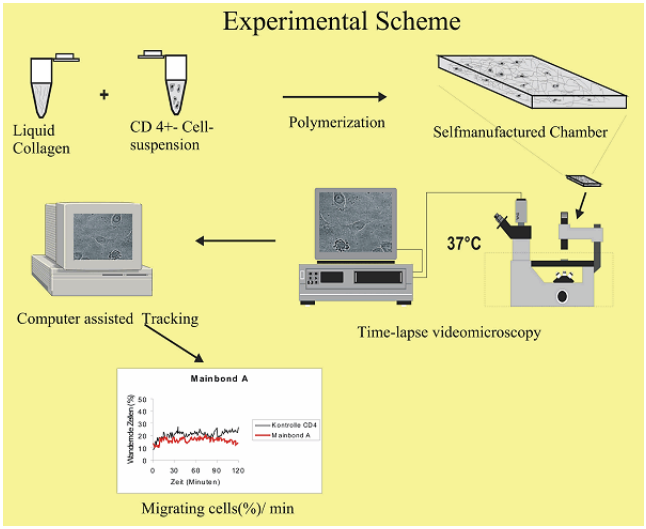
In vitro wound healing models predominantly are restricted to two-dimensionality. It was our intention to show, wether this three-dimensional in vitro model by evaluating the influence of different dental alloys and titanium on CD4+ lymphocyte migration, thus imitating one aspect of the immunological reaction in the marginal periodontal region may serve as a new in vitro-model of impaired response in periodontitis. Recently our group could show that spontaneous T-lymphocyte migration regulated by protein tyrosin kinase (PTK) being different from the protein kinase C (PKC) regulated induced type of migration in a 3-D collagen matrix model is modulated by foreign bodies such as dental alloys. However, little is known about the transition from the motile into a sessile state induced by "foreign bodies" placed subgingival or by impaired immune reponse in periodontally compromized patients. Specific T-cell activation requires stable cell-cell interaction.

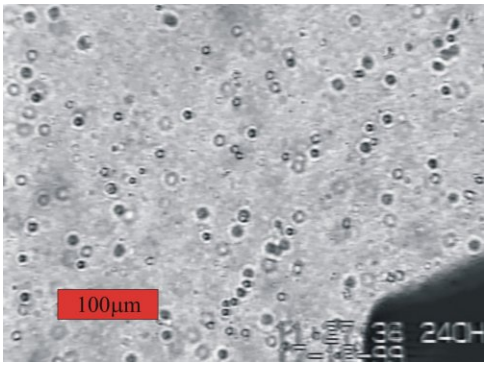
Objectives

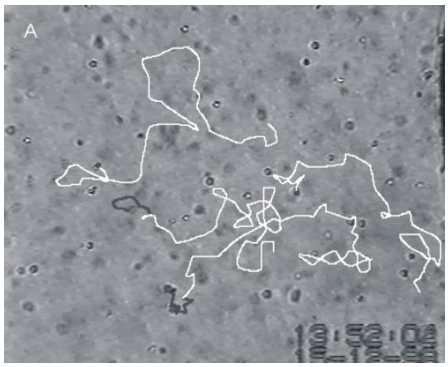
In our in vitro-model we tested the hypothesis of whether changes of locomotory behavior and receptor expression of CD4+ and CD8+ lymphocytes after induction of chemotaxis by different "foreign bodies" (precious dental alloys-pa, reduced precious dental alloys-rpa), cell-cell and mediated (via secreted signal substances) and interactions with different tumor cells could be ascribed to changes of the T-cell regulatory signal transduction of migration and receptor expression mimicing the influence of both either artificial tooth surfaces either impaired immune response on the development of periodontal diseases.

Material and Methods

Materials and Methods: The locomotion of immunomagnetically isolated human peripheral blood lymphocytes suspended in100 µl PBS and 200 µl type I 3-D collagen gels was recorded using time-lapse videomicroscopy as previously described. 30 cells for monitoring of cell migration were randomly chosen. Two hours of cell migration were reduced to two minutes by time-lapse videomicroscopy. The trials were repeated five times. Significance was tested applying the Mann-Whitney-U-test. Computer assisted cell tracking was analyzed as to the percentage of cells moving and to the velocity influenced by 2 dental alloys and with epithelial carcinoma cells from breast (MDA-MB-468), bladder (T24) and colon (SW480). CD25 as well as CD45R0 receptor expression of CD4+ and CD8+ lymphocytes governing cell-dental material with 2 different groups of dental alloys and direct and mediated cellular interactions with the three lines of carcinoma cells were assessed by FACS analysis after 24h of incubation.

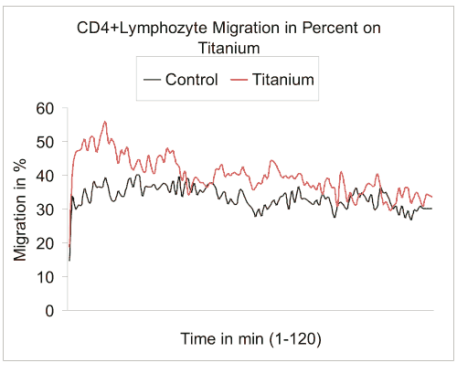


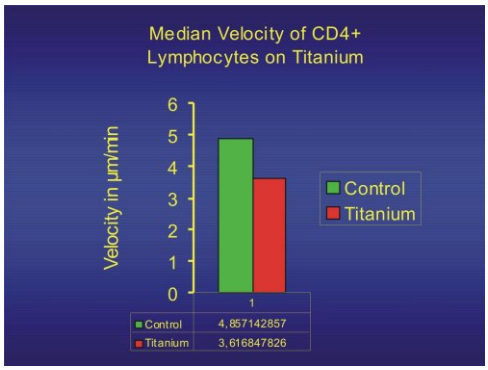




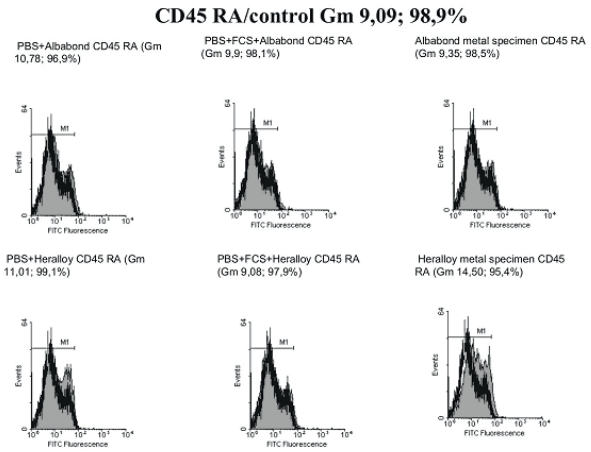
Selfconstructed chamber with a titanium specimen embedded in the collagen matrice.

This figure visualizes the pathways of four CD4+lymphocytes. We analyzed the percentage and the velocity of the cells mograting on different dental alloys and on titanium.





Titanium caused an enhancement of the percentage of the CD4+lymphocytes migrating.



Results

The medium percentage of CD4+ and CD8+ cells migrating was reduced on the 2 dental alloys tested versus the controls (pa 85,9%, rpa 39,7%) whereas being enhanced on titanium (110,9%). The velocity was reduced in all cases (pa=63,3%, rpa=39,4%, cp-Ti=28%) whereas percentage and velocity of T lymphocytes in direct and indirect cell interaction were enhanced. The results were highly significant (Mann-Whitney-U-test, $p<0.001$) in relationship to the method of dilution (PBS or serum) used. 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. The FACS analysis of the activation status of CD25 and CD45RO did not to show any differences between the tested groups and the controls influenced by dental materials, while showing more changes to activation in direct than in indirect cellular interaction especially concerning CD25RO compared to CD25 with all tumor cell species.

Conclusions

We presume that CD4+ and CD8+ lymphocytes are migrating in a 3-D collagen matrix migration model influenced by the eluated ion-profiles of the oxide material top layers in a concentration dependent manner. Thus the cell-material governed alterations of migration do not seem to be interdependent on the alterations of the expression of CD25 and CD45RO T lymphocyte cell receptors while enhanced migration due more to direct than to indirect cell interactions is coincident with changes in the status of activation of these receptors. This model is suitable for investigating further items of signal transduction pathways within cell migration and for the evaluation of modulating factors leading to impaired host response in immunologically compromized patients with periodontal diseases.

This Poster was submitted by Prof. Dr. Wolf-Dieter Grimm.

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