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On-line analysis of CAL72 cells on two different titanium surfaces in a perfusion micro-bioreactor

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Summary *Objectives*. The aim of the present experiment was to test a prototype microsensoric measuring system (micro-bioreactor) for the investigation of the biocompatibility of different titanium surfaces in a cell culture model.

Methods. Osteosarcoma cells of the cell line CAL72 were seeded onto titanium plates (10 mm × 10 mm × 1 mm) and inserted into the culture chamber of the micro-bioreactor. Titanium plates with two different surface topographies (machined and titanium plasma-sprayed [TPS]) were used for this pilot investigation. Plastic plates served as controls. The online-sensoric device of the micro-bioreactor allowed the continuous monitoring of the metabolism of the cells and the control of the culture conditions. Over a period of 17 h changes in O_2-consumption in the medium were measured by micro-electrodes and registered by the software of the system. The metabolic activity of the cells was calculated from the difference between the bypass and the chamber values. The cell proliferation and vitality were analyzed before and after the perfusion time in the micro-bioreactor. The cell morphology was studied using scanning electron microscopy.

Results. The cells on the machined surfaces showed the highest oxygen consumption after 15 h, after that it decreased. The cells on the TPS plates showed a lower oxygen consumption, which remained stable after 17 h. The highest oxygen consumption was seen with the cells on the control plastic plates. Concerning cell proliferation analysis, it could be shown that more vital CAL72 cells seeded onto TPS and plastic could be detected after the passage through the micro-bioreactor. Hence, the number of vital cells on the machined surface was reduced after the passage.

Significance. Within the limits of this experiment, the presented micro-bioreactor system could offer a valuable method to examine the dynamic interactions of cells and materials under defined in vitro experimental conditions. While the presented system is already successfully used in

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the ecological/ecotoxical field, its routine use for investigating dental materials on a cellular level has to be evaluated.
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Introduction

In the field of dental implantology, new materials and surface topographies are continuously developed and introduced in the market. The materials and topographies have to fulfil several requirements of which stability and biocompatibility are the most important. To date titanium has been the most successful and the most often used dental implant material [1–5]. Factors for success are their biological acceptance and integritability into the host tissue (biocompatibility), as well as the integritability into the system. The surface topography of dental implants plays a major role regarding the optimal interaction between the implant surface and the living tissues for generating a stable implant-tissue-connection [6–8]. The successful application of medical implant devices requires modern testing systems for evaluating the host tissue acceptance since the possibility exists that these materials release substances that could negatively influence the implant–host interaction (i.e. allergic reactions, non-integration). The intensity of such foreign body reactions depends among other factors on the chemical and functional biocompatibility of the material. At the stage of preclinical evaluation of biocompatibility, different in vitro and in vivo testing models are applied. In the field of dental implantology, animal experiments are quite often used for testing new materials or treatment techniques [9]. As standard in vitro investigational methods for the biological behavior and cytotoxicity of medical products, the Agar-overlay test, the filter test [10] and the MTT test [11] are applied. The in vitro study of cellular function is fundamental to several fields of application, extending from environmental and toxicological research to clinical diagnostics.

In the present investigation, a new in vitro system for testing dental (implant) materials in a cell culture was evaluated. It was the aim of the experiment to find out whether with the use of a micro-bioreactor and cell culture system, the biocompatibility of materials can be evaluated using parameters such as cell growth and metabolic functions. The micro-bioreactor allows the simultaneous measurement of the O_2-concentration and pH-values under constant temperature conditions (37 °C). These are important physiologic parameters for cellular activity which have been used in different 'biotests' for the description of the toxicity of substances [12,13]. At the moment, the presented system is used successfully in the field of ecotoxicology. This pilot investigation applies the perfusion micro-bioreactor in implantology for the first time, evaluating the metabolic behavior and growth of osteosarcoma cells of the CAL72 line on different titanium surfaces (topographies).

Materials and methods

Perfusion micro-bioreactor with integrated microsensors

The prototype of the micro-bioreactor used in this study was developed by Wolf et al. [14]. It consists of two micro-bioreactors including cell culture chambers as central units as well as microsensors for the measurements of the O_2-concentration, pH-value and temperature. The sensors were developed for different vital cells and tissues to simultaneously record representative metabolic functions and cell-biological parameters. The system is computer-controlled. The computer controls the perfusion of the micro-bioreactor as well as it coordinates, saves, and integrates the data of the metabolic parameters. For optical and microscopical purposes, the cell culture chambers have optical windows above and below. With the aid of a perfusion system, the cells in the chambers are continuously provided with culture medium. A so-called bypass serves for collecting control data. The passage through the cell culture chambers is closed at certain intervals and the medium passes through the bypass directly to the microelectrodes.

Culture model and titanium surfaces

As a culture model, the human osteosarcoma cell line CAL72 (DSMZ No. ACC 439) was used. The cells were seeded onto plastic plates and onto titanium plates (10 mm × 10 mm × 1 mm) with two different surface topographies [machined, titanium plasma-sprayed (TPS)] and inserted into the culture chamber of the micro-bioreactor. Before use, the titanium plates were cleaned with 99.8% ethanol,

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