Development of dynamic three-dimensional cell culture models for in vitro biocompatibility testing

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Introduction

Materials of synthetic and natural origin must fulfill certain criteria of safety and biocompatibility when used in implants or as constituents of tissue engineered products. Within the framework of the project „Biofabrication for NIFE“ our working group develops new methods to prove the innocuousness of chemical or biotechnological products for their future use in implants. Uncritical use in patients comprises e.g. a lack of cytotoxicity, low immunological and allergenic properties, sufficient durability after implantation, regenerative properties, anti-infectious properties and no carcinogenic or genotoxic effects. A combination of conventional test systems, as well as newly developed analytical methods, must be established and defined as standard operating procedures for use in preclinical tests. Conventional cell culture methods are based on two-dimensional (2D) static conditions, where cells grow in a monolayer on the plastic surface of a cell culture flask or in a multi-well microplate, placed in an incubator. Such cultivation conditions, however, are far away from the real in vivo situation, where the majority of the tissues in the human body are three-dimensional (3D) and cells are surrounded by other cells and the extracellular matrix. Another important cultivation strategy is cultivation under dynamic conditions, where cells are constantly supplied with nutrients, toxic metabolites are actively removed and cell sensitivity is affected by shear stress. Here we demonstrate our first results in the establishment of 3D cell cultures using different methods: cells growing in spheroids, cells growing in a collagen-elastin matrix and cells growing in a PEG-fibrinogen hydrogel. Moreover, first dynamic cultivations of 3D constructs in perfusion bioreactors were performed.

Biocompatibility testing within the framework of the Project „Biofabrication for NIFE“

3D cell culture for biocompatibility tests

The in vitro testing on 3D cell culture models, including cell lines as well as primary cells has an outstanding importance, since it stimulates the in vivo microenvironment at the site of implantation. Different approaches to create static and dynamic in vitro systems are investigated in order to obtain physiologically relevant, high throughput, low-cost and reproducible systems.

Figure 1. Different approaches are established to develop 3D cell culture models, e.g.: (A) cells growing in a collagen scaffold; (B) cell spheroids (microtissues), treated with different concentrations of ZnO nanoparticles; (C) PEG-fibrinogen hydrogel-based 3D cell culture

Biocompatibility testing under dynamic conditions using 3D cell systems in parallel perfusion bioreactors with integrated oxygen sensors

A dynamic in vitro testing system using 3D cell culture models was established for biocompatibility testing under physiologically relevant conditions. Cell growth, as well as cytotoxicity can be monitored online using optical oxygen sensors - here oxygen consumption rate serves as an indirect indicator of cell number. Extracellular acidification (pH change) can also be measured online with the help of optical sensors.

Figure 2. Perfusion bioreactor (A) and complete system (B) including peristaltic pump, bioreactor and optical oxygen sensors

Figure 3. Optical oxygen sensors

Outlook

Established three-dimensional cell culture models are tested now in static and dynamic conditions. Furthermore, additional optical and electrochemical sensors could be applied for online cell growth monitoring. Direct comparison of IC₅₀ values for different substances in 2D and 3D cell culture systems will be performed. Gene expression profiles of the cells cultivated in 2D and 3D models, as well as in static and dynamic conditions will be analyzed. This work is performed in cooperation with the Institute of Organic Chemistry (Prof. A. Kirschning), Department of Plastic, Hand and Reconstructive Surgery (Prof. P. Vogt). Further cooperation with the Institute of Immunology (Prof. R. Förster) is planned.

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