

## STRUCTOPLATE: A NEWLY DEVELOPED 3D-MICROSTRUCTURED SURFACE IN MULTIWELL TISSUE CULTURE PLATES

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**INTRODUCTION:** The currently available conventional tissue culture devices are not suitable to form tissue-like aggregates, that require high cell density. Therefore, the engineering of a new generation of substrates that enable cultured cells to grow at higher cell density and to maintain more *in vivo*-like cell-to-cell interactions is very important in order to obtain more reliable results *in vitro*. To evaluate the cell-compatibility and the usefulness of Structoplate as new substrate for routine cell cultures, the cell behaviour on 6 well and 24 well Structoplate was investigated.

**METHODS:** Primary isolated rat osteoblasts and rat chondrocytes, the macrophage cell line J774, the osteoblast cell line MC3T3-E1 and the fibroblast cell line 3T3 were used to examine the cell-compatibility of the newly developed Multiwell Structoplate (Integra Biosciences Holding AG, Acherfang, 6274 Eschenbach). Cells were added to the Structoplate (6 well and 24 well plates) at a density of  $5 \times 10^4$ ,  $10 \times 10^4$ ,  $1.5 \times 10^5$ ,  $2 \times 10^5$  and  $2.5 \times 10^5$  cells per well in 2 ml (for 24 well plates) or 5 ml (for 6 well plates) of their respective culture medium and allowed to attach at 37°C. For the measurement of cell attachment, cells were washed twice with PBS to remove non-adherent cells and the number of attached and viable cells was determined 4h after cell seeding with the MTT assay. To examine cell growth, cell density was determined 1, 2, 4, and 6 d after cell seeding using the MTT test and by counting the cell number. As a control, cells were plated onto 6-well tissue-culture plates (Costar) or 24 well plates (Nunc).

The content of collagen type I and Type II osteocalcin, and chondroitin sulphate was determined in ELISA tests. The alkaline phosphatase activity (ALP) was measured spectroscopically at 405 nm with p-nitrophenyl as substrate.

**RESULTS: Enhanced cell morphology:** The visibility under microscope was identical between Structoplate and conventional plates. The topography of the microstructures permits

unlimited observation of the adhered cells. which showed that the unique surface topography affected the cell spreading: cells seeded on Structoplate exhibited more rounded cell morphology than cells on conventional plates. Apart from macrophages, both primary isolated cells and cell lines built confluent cell multilayers.

### 6-fold higher cell densities on 24 well plates:

The cell densities of fibroblast cell line 3T3 and rat osteoblasts was measured 48 hours after seeding on 24 well Structoplate and conventional 24 well plates. Cell aggregation and cell detachment were found at higher cell densities only on conventional 24 well plates but not on Structoplate. Also, at high cell densities the number of attached cells was up to 6 fold higher on Structoplate compared to control plates.

**Phenotype and markers:** In addition to the strong attachment and high growth rate, osteoblasts preserve their specific phenotype. There were no significant differences in ALP activity, collagen type I, and osteocalcin produced by primary isolated rat osteoblasts.

**DISCUSSION & CONCLUSIONS:** The newly developed 3D-microstructured Structoplate provide the cultured cells with *in-vivo* like structures and enhanced surface area which results in 1.7 – 6 fold higher cell densities. The topography of the microstructures supports homogeneous spreading of the cells as well as the formation of cell multi-layers which results in improved phenotypes and stronger attachment to the growth surface. Structoplate demonstrated its ability to enhance cell cultures in various ways and may be a valuable alternative for biochemical coating of cell culture devices.