Importance of biomaterials testing prior to the construction of devices for *in vitro* techniques

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Introduction

Biomaterials testing is of high relevance in the medical sector, for example to verify the acceptance of artificial implants by the surrounding tissue. The Council Directive 93/42/EEC of the European Communities regulates the testing of medical devices for compatibility with contacting tissues. Recently, *in vitro* methods have gained more importance in the field of biomaterials testing. Here in turn, for the cultivation of cells it is necessary to exclude potential effects of the laboratory materials used on the viability of the cell cultures (Hiebl et al. 2010). Similarly, technical devices for *in vitro* techniques should be constructed by using materials that are proven to be harmless for the cell cultures.

Stainless steel and silicone are often used for laboratory equipment, due to their high durability and compatibility with cell and tissue cultures in general. However, our results highlight that different stainless steel and silicone types may show high variability in the degree of cytotoxicity, depending on their exact composition. Traces of toxic components might also be present on the surface of certain materials as a result of manufacturing processes (Anderson et al. 2004). Thus, biomaterials testing of all devices for cell and tissue cultures is advisable.

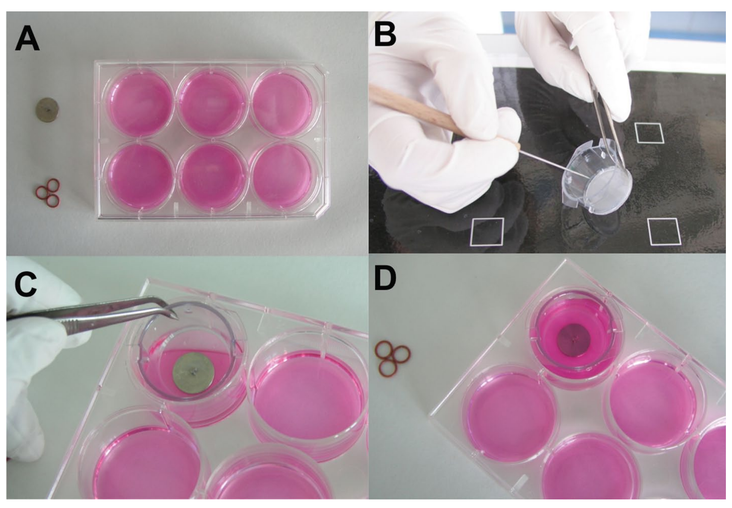
Scope of Study

We developed an easy and convenient approach that enables the reliable assessment of cytotoxicity of biomaterials both quantitatively and qualitatively. We further evaluated different cell lines (finite or immortal) for their suitability for biomaterials testing.

The information obtained was taken into consideration for the construction of the CULTEX® Radial Flow System (RFS), a device for the exposure of cell cultures to airborne substances at the air-liquid interface (Aufderheide et al. 2011).

The CULTEX® Radial Flow System (RFS). Potential cytotoxic effects of the materials used were excluded by biomaterials testing prior to the construction.

Material & Methods



A The cells (A549- immortal lung cancer cell line or IMR90 fetal lung fibroblast with finite lifespan) were seeded into the wells of a cell culture insert companion plate (6-well format). The supplied medium and cell seeding density was adapted to the needs of the particular cell line.

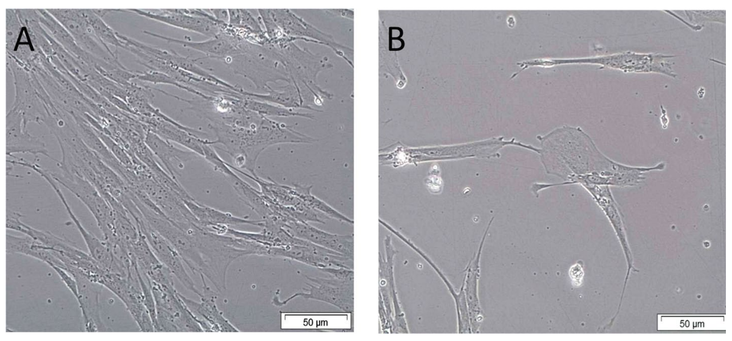
B The polycarbonate terephthalate (PET) membrane of commercial cell culture inserts was perforated with a needle.

C The test sample was placed onto the perforated membrane and the cell culture inserts were set in the wells of the companion plate.

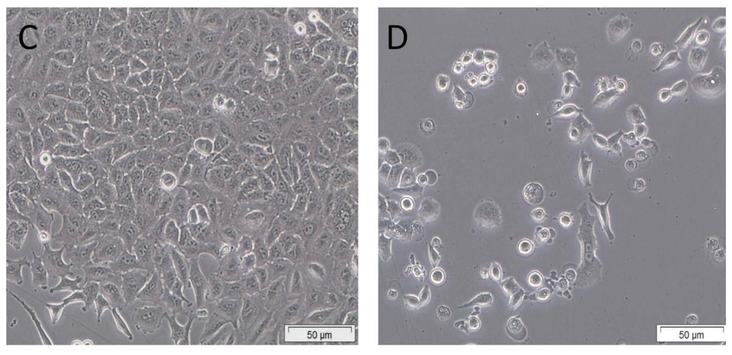
D The test sample was covered with cell culture medium. The perforated membranes facilitate the medium exchange between insert and well (indirect contact of test material and cell culture).

Qualitative Analysis

The cell growth and morphology was frequently checked by inverse microscopy. The cytotoxic potential of the material samples was evaluated by comparing cell cultures that have been cultivated in contact with the test material and control cell cultures.

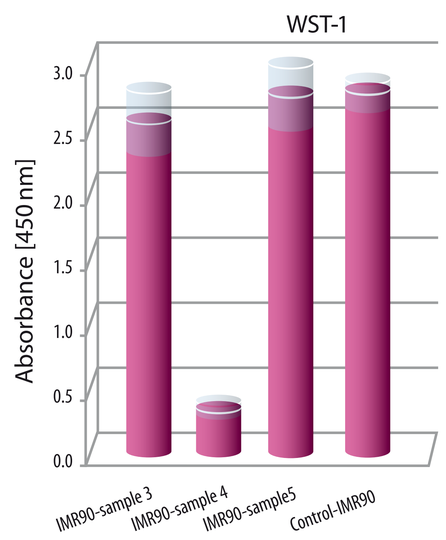


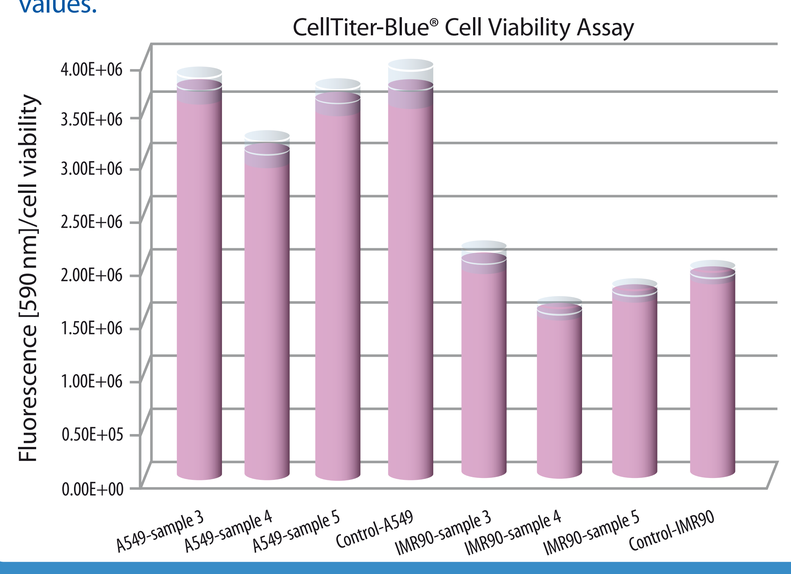
IMR90 cell cultures. **A**: Control culture; **B**: Cell culture cultivated in the presence of a test material. A cytotoxic effect was detected by comparing the cell growth and morphology to the control culture. This result was verified by A549 cell cultures (**C**: Control culture; **D**: Test culture).



Quantitative Analysis

The cytotoxic potential of the test materials was analysed quantitatively by measuring the cell viability after a cultivation time of several days. The cell viability was determined by different commercial assays like the WST-1 or CellTiter-Blue® Cell Viability Assay (Promega Corp.). A control culture (wells holding an insert but no material sample) was maintained for evaluation of the obtained values.





Results & Conclusions

The cytotoxic effects varied significantly between the different stainless steel and silicone types. Moreover, materials with similar compositions showed differences in their toxic potential when obtained from different suppliers potentially due to variable manufacturing processes.

The presented approach of biomaterials testing enabled the reliable assessment of cytotoxicity. Here, the cells were exposed to all leachable substances of the test material during the entire cultivation time and the material did not cause physical trauma to the cells.

In some cases, the quantitative analysis did not indicate a decrease in cell viability but the microscopical evaluation revealed a negative effect on the cells. This highlights the usefulness of applying both analysis methods.

The immortal cancer-derived cell line A549 did not show significant disparities in the sensitivity to cytotoxic substances compared to the fetal fibroblasts IMR90.

Materials testing is important when constructing devices for *in vitro* techniques, especially for long-term use and repeated applications, to preclude negative effects on cell viability and physiology.

Literature Cited

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