Evaluation of biomaterials using an *in vitro* test battery

A. Bruinink, F. Wein, M. Moczulska, V. Zell, M. Obarzanek-Fojt, M. Rottmar, X. Maeder-Althaus

EMPA-Materials –Science & Technology, Lerchenfeldstr. 5, 9014 St. Gallen, CH

INTRODUCTION: The proof of cytocompatibility (absence of cytotoxicity and presence of bioactivity) is a crucial step in developing new implant (and scaffold) materials and their surfaces. Currently, cytocompatibility is evaluated using ISO 10993-5 standards to prove that no toxic compound is released. Bioactivity is shown by seeding single cells of one cell type onto sample surfaces measuring at least cell adhesion, spreading and proliferation. The *in vivo* niche around the implant is however composed of multiple cell types which are subjected to complex interactions with each other in competing for the implant surface space. Furthermore, three-dimensional (3-D) tissues are contacting the implant. It is assumed that the clinical success and fate of the implant is ultimately determined by these interactions. So far this aspect is only taken into account by evaluating implant materials using animal studies. *In vivo* models remain extremely challenging due to the complexity of the system and the inability to individually elucidate the numerous mechanisms behind specific host responses particularly at the cellular level. Systematic approaches with a high number of material variation and multiple time-point evaluation are practically limited due to the cost, time and effort needed to conduct animal studies.

The aim of the present study was to define an intermediate step between the current *in vitro* and *in vivo* tests having the advantages of the *in vitro* environment while mimicking at least some aspects of the *in vivo* environment.

PROPOSED TEST BATTERY

The proposed test battery is shown in Fig. 1. Generally, going down in the test sequence each test of the test battery has an increasing degree of specificity and complexity. The first two tests evaluate the potential release of toxic components based on ISO 10993-5: extract test and ISO 10993-5: contact test. The subsequent 4 tests assess the bioactivity of the material by seeding cells directly on top of it. Evaluated is i) cell adhesion and spreading, ii) cell outgrowth out of a tissue-like cell reaggregate, iii) effects on cell proliferation and differentiation and iv) cell-cell competition/ interaction evaluating of each cell type of the co-culture cell proliferation and differentiation. The latter and final test has the potential to predict which cell type finally covers the implant surface. After each test promising materials are selected and tested in the next level. Materials that perform adequate in all test levels are thought to be optimal and ready for subsequent *in vivo* testing. We recently started to assess the strength of this test battery especially of 3-D reaggregate and cell-cell co-culture tests [1-2].

The proposed test battery has the potential to reduce time to market and cost per new developed implant that positively passes the human trials.

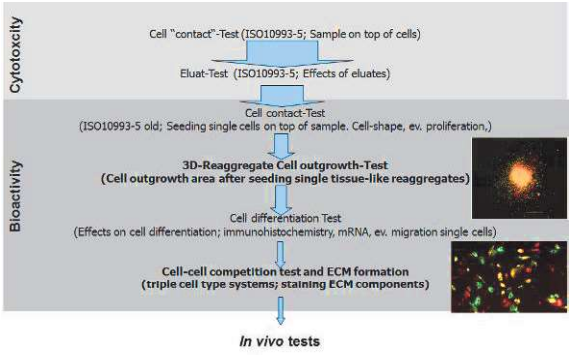

Fig. 1: The tests battery represents a sequence of in vitro tests with increasing complexity and costs. Each test focuses on a different aspect of the cell-material interactions. The arrow width illustrates the number of test materials entering the next test.

REFERENCES:

[1] M. Moczulska, M. Bitar, W. Swieskowski, A. Bruinink. Eur Cells Mat, 2010, 20 Suppl 1, 34.
[2] F. Wein, U. Tobler, C. Brose, J. P. Kaiser, A. Bruinink. Eur Cells Mat, 2010, 20 Suppl 1, 51.

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