

The Role of In Vitro Techniques in Tissue Engineering Development

C.J. Kirkpatrick

¹ *Institute of Pathology, Johannes Gutenberg University, Mainz, Germany*

INTRODUCTION: As the field of biomaterial research becomes more sophisticated, including the approach of tissue engineering, there has been a necessary expansion of the concept of biocompatibility to address not only the biosafety issue, that is, the exclusion of cytotoxic and other deleterious effects of biomaterials, but also the biofunctionality component, which concerns the fulfilment of the intended function of the applied biomaterial. Careful scrutiny of this concept leads to the conclusion that relevant test systems for biofunctionality must centre on human cells, studied under conditions relevant to the situation in the living organism for which the medical device has been constructed. Thus, progress in biocompatibility and tissue engineering (TE) would be inconceivable without the aid of *in vitro* techniques.

CURRENT DEVELOPMENTS:

Of paramount importance is proving the maintenance of the cell phenotype *in vitro*. Loss of essential characteristic functions of cultivated cells makes extrapolatory interpretations meaningless for the clinical situation. Until now much experimentation *in vitro* has concentrated on non-human cell systems in two-dimensional culture, very often with a view to excluding cytotoxic effects. For TE it is necessary to develop three-dimensional culture systems, in which, for example, confocal laser scanning microscopy with relevant immunocytological methods can greatly assist monitoring functional parameters [1,2], as well as co-culture systems [3] and dynamic cultures, as in bioreactors. This increased level of complexity is regarded as essential for a deepening of our understanding of biological mechanisms, without which a rational approach to TE design will not be possible.

Due to our interest in vascularization, much of our experimentation involves endothelial cells (EC) from microvascular sources as well as endothelial progenitor cells (EPC) from human peripheral blood. Among the biopolymers of interest are the silk protein, fibroin, in combination with collagen type I and chitosan-based scaffolds for bone TE. Examples will be given of experimental set-ups which enable cell functionality on the scaffold to

be studied both at the gene transcript and protein level, thus illustrating the possibility to make *in vitro* methods more *in vivo*-like. A further example is the use of co-cultures of osteoblasts and EC to understand how these two essential cell types for bone regeneration influence each other, especially in the interaction with a biomaterial scaffold. Our initial studies indicate that EPC and adult EC behave differently in their interaction with cells of osteoblastic phenotype.

In the field of drug- and gene-delivery we have established a co-culture model of the air-blood (alveolo-capillary) barrier of the human lung [3] with a view to using it to study the mechanisms of nanoparticle uptake and transport. This basic knowledge is required to enable targeting to the lung to treat pulmonary disease as well as to use the lung as a portal of entry to the systemic circulation to target, for example, cancer in other organs.

FUTURE DIRECTIONS & CONCLUSIONS:

There is increasing interest in understanding how the regenerative potential in various tissues and organs can be specifically targeted. Of particular significance is an understanding of how the so-called stem cell niche is controlled, as this forms the basis for rational targeting strategies, using, for example, drug- or gene-delivery systems. In addition, novel bioreactor technologies will continue to be vital to the field of tissue engineering, as will the application of nanotechnologies. In all of these areas of interest *in vitro* methodology is an important component in development and testing approaches. Nevertheless, it must be stressed that even if human cells are employed under optimal culture conditions, the problem of extrapolation to the *in vivo* state should not be underestimated.

REFERENCES: ¹ R.E. Unger, K. Peters, Q. Huang, et al (2005) *Biomaterials* **26**:3461-9. ² K. Peters, H. Schmidt, R.E. Unger et al (2005) *Molec Cell Biochem* **270**:157-66. ³ M.I. Hermanns, R.E. Unger, K. Kehe et al (2004) *Lab Invest* **84**:736-52.

ACKNOWLEDGEMENTS: The author wishes to express his thanks to the European Commission for various grants within the scope of its biomaterial programmes (esp. the NoE EXPERTISSUES), and the German Research Foundation (DFG; Priority Programme Biosystem 322 1100).