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The challenge of regulating angiogenesis in tissue engineered implants

C.J. Kirkpatrick

Repair lab, Institute of Pathology, Johannes Gutenberg University, Mainz, Germany

The success of tissue engineering (TE) implants will depend in large measure on the ability of the chosen system to provide an adequate oxygen supply to the cells involved, as practically all cell types in the human body (with the exception of cartilage) have an aerobic metabolism. Thus, it is important to develop relevant assays for human angiogenesis in order to better understand the underlying molecular mechanisms and to test new TE concepts for vascularization [1]. Cell cultures form an essential model system in studying vascular system-biomaterial interactions for TE applications, irrespective of whether a biomaterial scaffold or matrix will be pre-seeded with cells prior to implantation or the cell-material interactions are induced in vivo, for example by a suitable drug-delivery system to promote angiogenesis. The author will discuss in detail the underlying biological mechanisms and present data on human endothelial cell (EC) cultures, both as established cell lines and as primary isolated cells, in assays designed to unravel the mechanisms of vascularization of biomaterial matrices and scaffolds.

We have developed two-and three-dimensional assays using human EC of both macro- and microvascular origin (HUVEC, HDMEC, HPMEC). This assay system includes pro-angiogenic factors and suitable matrices (collagen I and fibrin) and promotes formation of lumen-containing vascular sprouts [2]. In order to test novel 3D scaffolds for TE we have performed detailed studies on the silk protein, fibroin, in the form of a fibre network [3]. Molecular biological methods, such as RT-PCR are employed to study the functional status of the cells. Seeding fibroin scaffolds with EC, followed by later addition of collagen I with fibrin to fill the scaffold interstices, permits extensive growth of EC on the network and vascular sprouting into the gel matrix, as studied using confocal laser scanning microscopy (CLSM). With such cultures we have shown that it is possible to establish in vitro assays to test the suitability of different biomaterial scaffolds for vascularization. RT-PCR has shown, for example, that the EC on the fibroin network are not activated [4]. Similar work is being performed on porous ceramics and metal surfaces.

Currently, co-cultures of EC and osteoblasts are being established to simulate more closely the in vivo situation during bone regeneration. These models offer possibilities to study other wound healing forms, such as for skin regeneration (EC together with keratinocytes and dermal fibroblasts). In addition, studies with endothelial progenitor cells (EPC) from human blood are also being performed, as this gives the possibility of employing autologous cells for vascularization. The latter probably represents the most attractive possibility for vascularisation of TE implants, although it must be stressed that many fundamental biological issues with respect to endothelial lineage differentiation still remain to be clarified. In particular, it is still unclear how pure the EPC population must be to achieve maximum success. Thus, it is possible that heterotypic cell interactions might be essential to regulate lineage differentiation. A further issue concerns the possible activation of immunogenic signals (even) in autologous cells induced by the artificial situation of in vitro cultivation.

In conclusion, as TE applications approach possible clinical use, the requirement for detailed knowledge on how human vascular cells interact with biomaterials will become increasingly important, as vascularisation and control of the inflammatory and healing responses are prerequisites for clinical success.

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