Endothelial cell migration and aggregation in response to hypoxia-induced signalling

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Introduction

The vascularisation of any graft, engineered implant or injury site is a key factor for optimal repair and regeneration. New blood vessel formation is a physiological response to tissue hypoxia, through upregulation of angiogenic factor signalling. We engineered cell-mediated hypoxia in a convenient cell type, human dermal fibroblasts (HDFs), to form a population of Hypoxia-Induced Signalling (*HIS*) cells and showed that *HIS* responses by HDFs induce endothelial cell (EC) migration and tubule formation both *in vitro* and *in vivo*.

Materials and Methods

Capillary-like structure formation by EC's (HUVEC'S) in response to the *HIS* response by HDF's was measured in a 3D collagen matrix assay. This assay tested EC migration (up to 1 cm) and aggregation towards a HIS cell source (fig.1). EC capillary-like structure (CLS) formation was monitored over 14 days. Constructs containing *HIS* cells were also seeded *in vivo* and the functionality of invading vessels was verified by real-time monitoring of O_2 in the core of implanted constructs.

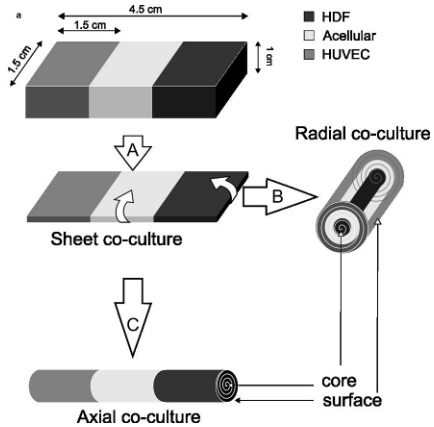

Fig. 1 Schematic showing construction of 3D collagen matrix with spatially positioned cells. Maximal protein was found in the core.

Results

By positioning *HIS* cells and ECs in distinct locations within 3D collagen constructs, we were able to quantify CLS formation by EC's in response to *HIS* cells, which induced directional EC sprouting *in vitro* (fig.2). Furthermore, depots of *HIS* cells, positioned in the core of 3D collagen constructs could direct host vessels deep into the matrix within 1 week *in vivo* implantation in rabbits.

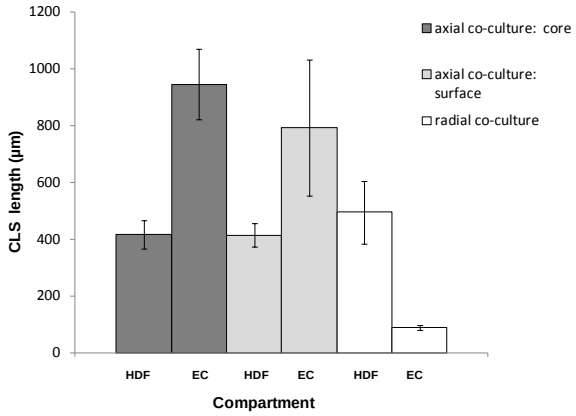

Fig. 2. Graph showing CLS formation mainly in the EC/HUVEC region of the axial co-culture. In the radial co-culture this CLS formation was inhibited due to decreased diffusion of angiogenic proteins through multiple collagen layers.

Discussion and Conclusions

These findings unravel the angiogenic potential of *HIS* cells with important implications for *in vitro* tissue modelling, as well as devising implant vascularisation strategies and potent angiogenic therapies for ischaemic diseases.

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