Influence of fiber size of silk fibroin scaffolds on endothelial cells response

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Statement of Purpose

We are currently testing the influence of nano- and micrometric silk fibroin nets on molecules involved in endothelial cell adhesion, polarization and migration. This is important in order to understand how the fiber size might influence endothelial cell function in terms of adhesion, as well in terms of vascularisation of tissue engineered constructs or endothelialisation of vascular stents.

Methods:

Two different fibroin sources were used to produce nets, "White" (PN) and "golden" (PU) cocoons (Bombyx mori). For preparation of micronets please refer to (2). Nano-metric nets were prepared by electrospinning. In brief, after cocoon degumming, fibroin was dissolved in LiBr 9.3M and dialyzed against distilled water. Fibroin water solutions were then lyophilized and the obtained powder dissolved in formic acid (12% by weight). After lyophilization, molecular weight and aminoacidic composition of the PN and PU fibroin were determined.

We are using quantitative real time PCR, as well as confocal fluorescence microscopy to study the expression and the distribution of molecules involved in endothelial cell adhesion and polarization on the silk nano- and micrometric nets. A set of relevant molecules is currently under investigation in different types of endothelial cells, such as HUVEC, resembling the macrovascular endothelial cell type, and outgrowth endothelial cells (OEC)(*1, 2*), which might serve as a potential source of autologous cells from an adult donor. Outgrowth endothelial cells were isolated from human peripheral blood buffy coats, expanded and analysed for their differentiated endothelial phenotype. OEC express several marker such as CD31, VE-cadherin, vWF, CD146, CD34 and caveolin-1 throughout their expansion. These cells have previously been shown to retain the differentiated and functional phenotype when cultured on micrometric fibroin scaffolds. Endothelial cells were first grown on coverslips. Focal adhesion molecules such as vinculin, paxillin or focal adhesion kinase and the cytoskeleton were investigated by immunofluorescence using standard immunofluorescence protocols at several time points after inducing endothelial cell migration and wound healing processes by making linear scratch defects in the monolayer. Other molecules relevant for endothelial cell polarization and response of endothelial cells on growth factors such as caveolin-1 and its phosphorylated form were investigated in parallel. On the basis of these results under 2-D conditions the molecules were investigated when cells are seeded and cultured on fibronectin coated fibroin scaffold material with nano- or micro-sized fibers. After 7 days of culture constructs were investigated using immunofluorescence or quanitative real time-PCR.

Results/Discussion:

Endothelial cells HUVEC and OEC adhered to and formed confluent endothelial cell layers on both the micro and the nanometric fibroin scaffolds with differentiated endothelial cell contacts. Furthermore endothelial cells form distinct focal contacts with the single nanofibers (Figure 1) and seem to be more polarized compared to the micrometric net. In ongoing experiments we are establishing a panel of markers of endothelial cell adhesion and polarization for a more quantitative analysis of endothelial cell function on nano- and micrometric nets.

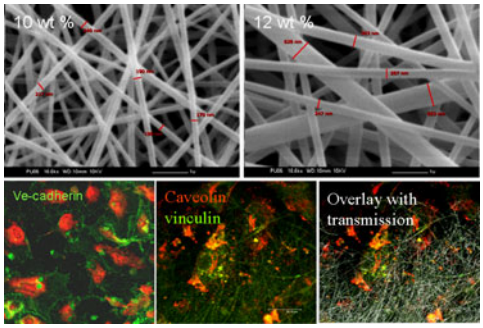

Figure 1
Outgrowth endothelial cells OEC grown on nanometric (SEM of pure material, upper row) fibroin silk scaffolds form endothelial cell contacts (depicted for VE-cadherin) and form distinct focal contacts at the level of the single nanofibers.

Conclusions:

Endothelial cells grown on nano-metric nets form distinct focal contacts with individual nanofibers but reveal a polarized morphology with a distinct distribution of molecules involved in endothelial cell polarization. Further studies evaluating potential differences in cell adhesion molecules are under way.

References:

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2. S. Fuchs, A. Motta, C. Migliaresi, C. J. Kirkpatrick, *Biomaterials* **27**, 5399 (Nov, 2006).

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