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Influence of cell source and adhesion substrate on growth factor responsiveness in primary endothelial cells

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Abstract

In vitro testing is an important step in the development of new implant materials. With regard to applicability of results obtained in vitro to later preclinical and clinical tests, primary cell culture is best suited to yield meaningful results in terms of biocompatibility of newly developed biomaterials. However, unlike cell lines primary cells cannot be propagated to infinity, a fact that necessitates a fairly frequent change of cell source.
We are using primary human umbilical vein endothelial cells (HUVECs) to test surface-functionalized polymers for their cell stimulatory potential. In addition to polymers, polystyrol and glass are parallel-tested as reference substrates. Cell viability and proliferation tests were done consecutively in the same microtiter plate.
Here we present data on the variability of stimulation of HUVECs from six different donors with vascular endothelial growth factor (VEGF) which was in various ways coupled to a polymer surface. In 24 individual measurements of cell proliferation, the extent of stimulation through VEGF ranged from 0.71 to 3.5-fold. When comparing different reference substrates, same-origin cells could be stimulated from 1.52 to 1.65-fold or 0.71 to 3.11-fold, respectively. On the functionalized polymer itself, cell viability and proliferation could be stimulated up to 0.76 and 2.07-fold, respectively.
The degree of variability in growth factor responsiveness is remarkable. This finding implies that for in vitro testing on primary cells in general, and for biomaterial testing in particular, a representative number of both cell batches and reference materials should always be included.

1 Introduction

In vitro testing is an important step in the development of new implant materials. With regard to applicability of results obtained *in vitro* to later preclinical and clinical tests, primary cell culture is best suited to yield meaningful results in terms of biocompatibility of newly developed biomaterials. However, unlike cell lines primary cells cannot be propagated to infinity, a fact that necessitates a frequent change of cell source. This is cause for some concern regarding reproducibility of results.
In our laboratory, we are using primary human umbilical vein endothelial cells (HUVECs) to test surface-functionalized polymers for both their biocompatibility and cell stimulatory potential. These experiments are designed to provide insights into the polymers' feasibility for use in cardiovascular implants.

2 Methods

2.1. Polymer surface modification

To improve the endothelialization of poly(ε-caprolactone) (PCL) intended as scaffold material for bioartificial vessel prostheses, the polymer surface was activated with functional groups. Via oxygen (O_2) plasma/aminopropyltriethoxysilane (APTES), hexamethylenediamine (HMDA) and 4,4'-methylene-bis(phenyl isocyanate) (MDI)/ water, terminal amino groups were generated. Alternatively, through O_2 plasma activation, terminal hydroxyl groups were generated [1].
Then, immobilization of vascular endothelial growth factor (VEGF) was performed either by adsorption or by employing 1-Ethyl-3-[3-dimethyl-aminopropyl]carbodiimide (EDC) and N,N-disuccinimidyl carbonate (DSC) as cross-linkers (figure 1). The various surface modifications were confirmed by contact angle or fluorescence measurements, X-ray photoelectron spectroscopy (XPS) and infrared spectroscopy.

2.2. Primary cell culture

Human umbilical vein endothelial cells (HUVECs) were cultured, harvested after 3 to 6 passages, and seeded onto discs of PCL and its respective modifications in microtiter plates. After incubation for 96 hours, cell viability was tested with the Cell Quanti Blue kit (BioAssay Systems). Subsequently, relative rates of proliferation were measured on the same cells employing BrdU proliferation assay (Roche Diagnostics).

3 Results

3.1. VEGF effect on reference substrates

The first tests of the effect of VEGF on HUVECs with three different reference materials produced a wide range of relative stimulation (image 1). Of 57 individual measurements of cell proliferation in total, 63.1% yielded a VEGF-induced stimulation of more than 130% compared to unstimulated cells.

The degree of variability is independent of the substrate presented to the cells, but failure of stimulation is most frequently associated with thermanox (34.7% of tests on this substrate).

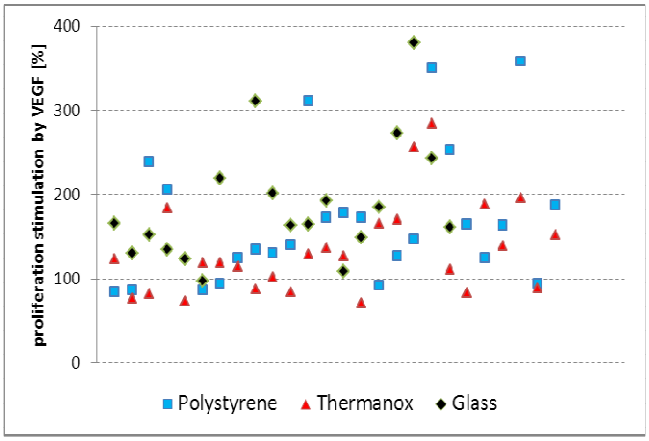

Image 1 Scatter plot of 24 individual proliferation tests with HUVECS from 8 different donors growing on 3 different reference substrates.

As shown in image 2, cell stimulation through VEGF on polystyrene varied from 1.36 to 3.74-fold for viability, and from 1.27 to 3.5-fold for proliferation between HUVECs donors, compared to the no-stimulus control.

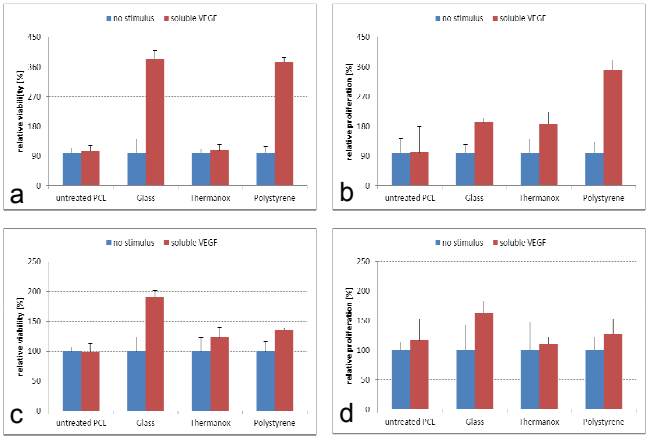

Image 2 VEGF-stimulated cell viability (a, c) and proliferation (b, d) of HUVECs from two different donors. Top panel: donor 1, bottom panel: donor 2.

3.2. VEGF effect on PCL

Poly(ε-caprolactone) (PCL) was either activated with functional groups or modified through salt leaching, a process that increases the surface that cells can adhere to. When cell viability on functionalized PCL was measured (image 3a), there was consistent stimulation of HUVECs with VEGF regardless of the state in which it was presented (soluble vs immobilized). The effective increase was slightly lower with the immobilized VEGF, though the difference to the soluble growth factor was not significant.

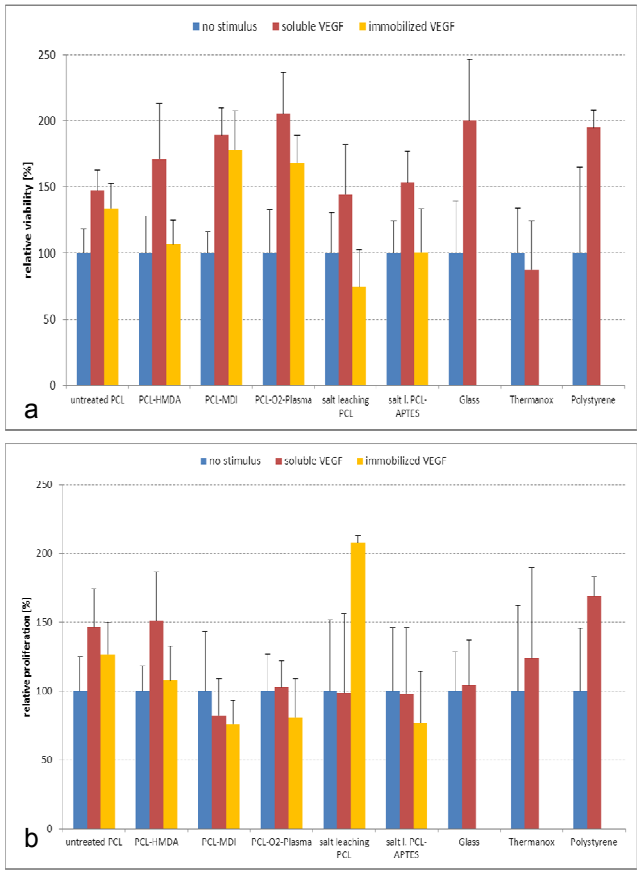

Image 3 Influence of adhesion substrate and PCL surface functionalization on VEGF-stimulated cell viability (a) and proliferation (b) of HUVECs (donor 3). VEGF was either immobilized on the polymer surface by adsorption prior to the seeding of cells (immobilized VEGF), or added to the cell culture medium thereafter (soluble VEGF).

A different picture was seen in the proliferation tests; here the effect of VEGF was much less consistent, with no stimulation seen on some substrates (image 3b).

3.3. VEGF effect on sPCL-A

Star-shaped poly(ε-caprolactone) with terminal acrylate groups (sPCL-A) was subsequently used as a substrate (image 4). This derivative of PCL has improved mechanical properties and degrades faster than PCL itself. The sPCL-A was activated and then VEGF was covalently coupled through EDC/NHS or DSC. The small difference seen in the PCL experiments between VEGF presented to the cells immobilized on the polymer and given directly into the culture medium was diminished or even reversed (image 4a and d) on sPCL-A. As the relative proliferation of the cells is not enhanced by VEGF in the polystyrene control for the second donor (d), the marked stimulation seen on sPLC-A/O_2-Plasma/DSC must probably be dismissed as a false positive.

As with PCL, the VEGF-mediated stimulation of cell proliferation was less consistent than that of relative viability of HUVECs.

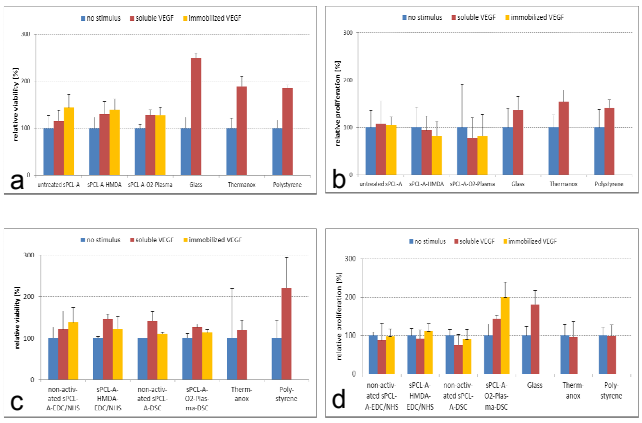

Image 4 Influence of surface functionalization of sPCL-A on VEGF-stimulated cell viability (a, c) and proliferation (b, d) of HUVECs. Non-activated controls had VEGF bound to the polymer surface through adsorption (c, d). Panels (a) and (b) represent cell donor 4, panels (c) and (d) represent cell donor 5.

4 Conclusion

The viability and proliferation data demonstrate pronounced variation of VEGF stimulation with cell donor. While endothelial cells on the whole could be well stimulated by VEGF both in soluble form and coupled to the polymer surface, the differences in the extent of that stimulation between substrates are considerable. This is particularly remarkable as it is also true for the control substrates, where in some cases no VEGF-mediated stimulation was observed. Overall, however, the growth factor was able to spike both cell proliferation and viability in HUVECs growing on a variety of substrates. The scale of that stimulation in all cells growing on the functionalized polymer surface was consistently lower than in those on the polystyrene control.

In conclusion, the concern for reproducibility of data obtained through *in vitro* testing of primary cells remains, as there is great variation with cells from different donors. This problem can only be alleviated by thorough study design including a suitable reference material and performing the tests over a representative number of cell isolates.

5 Acknowledgement

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6 Reference

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