

VEGF AND PDGF-BB INCREASE NEOVASCULARISATION INTO A HEPARIN-COATED POLYURETHANE

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INTRODUCTION: Spontaneous endothelialisation of a synthetic vascular graft is a prerequisite for long-term patency particularly in peripheral replacements. Transanastomotic endothelialisation is precluded in humans, therefore another source of endothelial cells would be the microvasculature surrounding the graft. As this would require transmural migration of the capillary derived EC's, we have developed a macroporous polyurethane graft. We now report on derivatisation of the surface of this porous polyurethane with proangiogenic Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor-BB (PDGF-BB) to stimulate neovascularisation and transmural ingrowth of capillaries.

METHODS: Polyurethane disks (5.4mm diameter, 2mm thick) with well-defined open porosity (82% porosity, $157\pm 1\mu\text{m}$ pores) were produced by a variation on the phase inversion technique using pre-packed spherical microbeads as porogens. Heparin was subsequently attached covalently to the PU via Acrylic acid-diamine spacers. VEGF₁₆₅ and PDGF-BB were passively adsorbed separately and in combination onto the heparinised surface at 4°C. The optimal loading and period of release was determined by in vitro elution assays using VEGF/PDGF-BB ELISA. Heparinised and non-heparinised discs containing different combinations of growth factors were implanted subcutaneously in Wistar rats for 10 days. Vascular density was assessed on cross-sections using image analysis after α -CD31 immunocytochemistry and reported as vessels/ μm^2 .

RESULTS: VEGF ELISA showed, that a maximum of 1.2 μg VEGF₁₆₅ (10% of the loading dose) was still adsorbed per disc after 3 washes in PBS and took 48 hrs to elute completely at 37°C. 3.5 μg PDGF-BB loaded onto PU discs was still adsorbed after 3 washes in PBS and only 5.9% of PDGF-BB was eluted after 7 days. Elution in 1M NaCl/PBS/0.5%BSA confirmed that 91.44% of the loaded PDGF-BB dose was still bound to the PU

discs. Vascular density of the PU discs after 10 days subcutaneous implantation in rats was significantly increased by heparin surface modification versus non-coated control discs (66.58 ± 1.76 vessels/ mm^2 vs. 118.27 ± 6.96 vessels/ mm^2 ; $p<0.05$). There was further a significant increase in vascular density by adding PDGF-BB 3.6 μg (138 ± 4.77 vessels/ mm^2), VEGF 12.5 μg (141.12 ± 6.49 vessels/ mm^2) and VEGF 12.5 μg plus PDGF-BB 1.8 μg (149.31 ± 12.29 vessels/ mm^2) onto heparin-coated PU discs.

DISCUSSION & CONCLUSIONS: Heparin as a delivery system for the controlled release of growth factors has been reported by passive addition to hydrogel matrices, by non-covalent as well as by covalent immobilization within different matrices [1]. In vivo delivery of VEGF in combination with heparin within a gelatin gel to a graft material has been previously reported by Masuda et al. [2]. VEGF immobilized in a heparin containing collagen matrix was described recently by Steffens et. al [3]. We report for the first time however, to our knowledge, on the direct, covalent attachment of heparin onto the biomaterial polyurethane without the need of an ingrowth matrix. The staged delivery of VEGF and PDGF-BB due to different affinities to the heparin-coated surface might further be a useful tool for future applications in the field of tissue engineering. The positive outcome suggests that this approach may be useful in the endothelialisation of porous synthetic vascular grafts.

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