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# Controlled release of vascular endothelial growth factor using poly-lactic-co-glycolic acid microspheres: In vitro characterization and application in polycaprolactone fumarate nerve conduits

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a potent angiogenic stimulator. Controlled release of such stimulators may enhance and guide the vascularization process, and when applied in a nerve conduit may play a role in nerve regeneration. We report the fabrication and in vitro characterization of poly-lactic-co-glycolic acid (PLGA) microspheres encapsulating VEGF and the in vivo application of nerve conduits supplemented with VEGF-containing microspheres. PLGA microspheres containing VEGF were prepared by the double emulsion–solvent evaporation technique. This yielded 83.16% of microspheres with a diameter <53 μm. VEGF content measured by ELISA indicated 93.79 ± 10.64% encapsulation efficiency. Release kinetics were characterized by an initial burst release of 67.6 ± 8.25% within the first 24 h, followed by consistent release of approximately 0.34% per day for 4 weeks. Bioactivity of the released VEGF was tested by human umbilical vein endothelial cell (HUVEC) proliferation assay. VEGF released at all time points enhanced HUVEC proliferation, confirming that VEGF retained its bioactivity throughout the 4 week time period. When the microsphere delivery system was placed in a biosynthetic nerve scaffold robust nerve regeneration was observed. This study established a novel system for controlled release of growth factors and enables in vivo studies of nerve conduits conditioned with this system.



## 1. Introduction

Nerve regeneration requires cells, extracellular matrix, growth factors and a complex interplay between the three. When a nerve defect is repaired with a single lumen conduit these components are not present. Introduction of growth factors (GF) or cells into a conduit would encourage axon growth in tissue engineering implants [1]. Most studies have focused on application of GF with neurotrophic effects. Since in the process of nerve regeneration, especially that after nerve graft repair or conduit repair, revascularization precedes regeneration the current study aims at the delivery of pro-angiogenic GF to the regenerating nerve site. Pro-angiogenic GF promote angiogenesis, leading to increased transportation of oxygen and nutrients to the nerve tissue. One important pro-angiogenic GF is vascular endothelial growth factor (VEGF). VEGF is a homodimeric glycoprotein that increases microvascular permeability, stimulates the proliferation and migration of endothelial cells, and promotes angiogenesis [2–4]. VEGF also directly affects neurons and glial cells by inhibition of apoptosis, promotion of survival and stimulation of neurogenesis. The angiogenic effect of VEGF on vascular remodeling may play an important indirect role in nerve regeneration.

The mode and timing of GF delivery is as important as the GF itself. Two major strategies have been used to administer angiogenic growth factors: application of exogenous recombinant human protein and gene transfer to induce endogenous secretion of the target growth factor [2–5]. There has been some success in pre-clinical animal models and initial clinical trials of pro-angiogenic GF delivery [6–8]. However, double-blind clinical trials with large cohorts of patients failed to show the efficacy of intravenous infusions of recombinant human VEGF or VEGF gene transfer ther-

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