# Engineering of a Human Endothelialized Connective Tissue and Evaluation of the Angiogenic Potential of the Neurotrophic Factors NGF, BDNF, NT3 and GDNF

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## Introduction

The implication of NGF in both nervous and vascular systems raises the question whether other neurotrophic factors can also be angiogenic. The purpose of this study was to investigate if other neurotrophic factors could have an angiogenic effect in vitro. To analyze the effect of neurotrophic factors on angiogenesis, we used an endothelialized reconstructed connective tissue model (ERCT) in which a human capillary-like network was allowed to organize and mature.

## Materials and Methods

The ERCT was produced from a collagen-chitosan sponge seeded with human dermal fibroblasts and endothelial cells isolated from umbilical cord or from newborn foreskin (1,2). The recombinant neurotrophins were mixed in the culture medium 17, 19 and 21 days after the seeding of the cells. The tissue biopsies were taken at day 31. The capillary-like nertwork was shown by PECAM immunofluorescent staining on 5 µm thick tissue cross sections. This staining were numerized and used to measure the depth of migration of the capillaries in the ERCT. The number of capillaries was determined by observations of 5 µm thick tissue cross-sections stained with Masson's trichrome.

## Results

We demonstrated that when 10 ng/ml of NGF, 0.1 ng/ml of BDNF, 15 ng/ml of NT-3 and 50 ng/ml of GDNF were added to the ERCT, a major increase from 40 to 80% in the number of capillary-like tubes was observed. An increase was also observed when a similar study was done using an ERCT made of human microvascular endothelial cells. For NGF, BDNF and NT-3, this angiogenic effect was mediated through the Trk receptors, since TrkA and B were demonstrated to be expressed by endothelial cells, and their angiogenic effect was abolished after a treatment with K252a, an inhibitor of TrkA and TrkB.

## Discussion and Conclusions

We generated an endothelialized reconstructed connective tissue that allowed for the study of the role of neurotrophins in angiogenesis in a very physiological environnement. This is the first in vitro demonstration of a direct angiogenic effect of neurotrophic factors and of the angiogenic potential of NT-3 and GDNF, the later belonging to another family of neurotrophic factors.

## References

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## Disclosures

The authors have nothing to disclose.