Restoration of Endothelial Cell Function in Ischemic Tissues for Accelerated Vascularization

Hong Niu, Zhaobo Fan, Zhaobin Xu, Haichang Li, Jianjun Guan,
Department of Materials Science and Engineering, The Ohio State University

Introduction: Critical limb ischemia is a severe peripheral artery disease characterized by low blood perfusion and degenerated skeletal muscle. Quick vascularization to restore blood perfusion is the primary goal for CLI treatment. However, there is no efficient treatment currently available. One of the major reasons is that functions of the endothelial cells are impaired in the ischemic limbs. This is the result of upregulated TGFβ decreasing endothelial cell proliferation and migration [1]. Thus, inhibiting TGFβ signaling pathway represents an approach to achieve fast angiogenesis. This work aims to: 1) develop a TGFβ Receptor II (TGFβRII) binding peptide that can inhibit TGFβ from binding endothelial cells; 2) create a novel drug delivery system capable of releasing the peptide and angiogenic growth factor bFGF, and 3) evaluate the efficacy of the delivery system in promoting fast angiogenesis using mouse ischemic limb model.

Materials and Methods: A thermosensitive, injectable and degradable hydrogel was used as the carrier for peptide and bFGF. The hydrogel was synthesized by free radical polymerization of N-isopropylacrylamide, 2-hydroxyethyl methylmethacrylate, acrylate-polylactide, and N-Acryloxysuccinimide. The core-shell microspheres with hydrogel as shell and ECG and bFGF as core were fabricated by electrospraying. Human Cardiac Microvascular Endothelial Cells (HMVECs) were used to determine the binding affinity, specificity and competitive binding of the peptide to TGFβRII. HMVECs were also cultured for cell proliferation and migration study. 3D model was created using collagen gel with the HMVECs to evaluate the effects of peptide and bFGF on the endothelial cell tube formation. The role of the peptide and bFGF in TGFβ pathway was investigated by western blot. Wild type C57BL/6 mice were used for ischemic limb models.

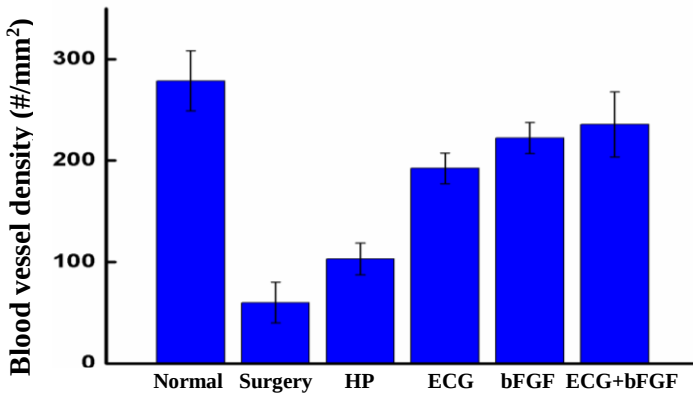

Figure 1. Blood vessel density of ischemic limbs 4 weeks after implantation of microspheres with core of HP only, ECG/HP, bFGF/HP, and bFGF/ECG/HP. Non-injection group and open surgery only group were used as controls.

Results and Discussion: The developed peptide can specifically bind TGFβRII with higher affinity than TGFβ. The migration study showed that peptide significantly improved HMVEC migration in the presence of TGFβ. The dsDNA results demonstrated that the peptide and peptide/bFGF promoted HMVEC growth while TGFβ severely delayed growth. The peptide and bFGF can gradually release from the microspheres for 4 weeks. The released peptide/bFGF stimulated tube formation when examined using the 3D collagen model seeded with HMVECs. the Western blot results showed that peptide/bFGF inhibited Smad2/3 phosphorylation in HMVECs. After injecting into ischemic limbs for 4 weeks, blood vessel density was significantly increased compared to control groups (Figure 1).

Conclusions: We have developed a novel TGFβ Receptor II binding peptide that can effectively inhibit TGFβ signaling pathway to restore endothelial cell function. The microsphere-based peptide and bFGF release system significantly promoted angiogenesis in ischemic limbs.

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References: [1] Maroni D, Davis J S. TGFB1 disrupts the angiogenic potential of microvascular endothelial cells of the corpus luteum[J]. J Cell Sci, 2011, 124(14): 2501-2510.