Peripheral vascular disease (PVD) affects ~8 million people per year in the USA alone, and causes significant morbidity and mortality worldwide. Amputation remains the definitive treatment for severe PVD, despite the numerous negative effects the loss of a limb can have for a patient with PVD.1 Recent advances in molecular and cellular therapeutics offer the possibility that therapeutic angiogenesis may improve patient outcomes and improve the standard of care for patients with PVD.1,2 Gene therapy approaches have been developed to increase neovascularization in models of critical limb ischaemia (CLI) by expressing gene(s) associated with angiogenesis. These approaches have demonstrated much promise at the preclinical level, and have led to numerous efficacy and safety studies with a variety of therapeutic genes. Clinical gene therapy studies have been challenged by the relatively short duration of gene expression in some systems, as well as by the suboptimal distribution of gene expression, and have failed to demonstrate a significant benefit of gene therapy over placebo.2

Cell-based therapy, as proposed in the report by Toyama et al.3 in this issue, offers an alternative to gene therapy that may improve efficacy and the therapeutic outcome through the implantation of living patient-derived cells into diseased tissue to express a variety of angiogenic factors with little or no immune response. Preclinical studies in animals have demonstrated that the implantation of bone marrow-derived mononuclear cells (BM MNCs) into ischaemic limbs is effective in increasing the expression of numerous angiogenic factors such as basic fibroblast growth factor, vascular endothelial growth factor, and angiopoietin-1 in ischaemic tissue and ultimately promotes vessel formation.4,5 Based on positive results in animal studies, several clinical trials were initiated.5–7 These trials demonstrated the safety of intramuscular injection of BM MNCs for patients with CLI and resulted in improvements in limb status5 and amputation-free survival6,7 in many patients. Despite these overall positive outcomes, cell treatment was unsatisfactory in a subset of patients with atherosclerotic risk factors or other chronic disease.8 It has been noted that the effectiveness of cell therapy is limited in patients with coronary artery disease, vascular disease, old age, diabetes, increased cholesterol, or hypertension.8,9 Most important to the treatment of CLI, the yield, migration, and neovascularization capacity of the circulating angiogenic cells (CACs) isolated from patients with atherosclerosis is significantly reduced compared with those from healthy subjects.8,9 Pretreatment of therapeutic cells ex vivo prior to implantation may increase the expression of angiogenic factors and improve the efficiency of cell therapy for chronically ill patients.8

To increase the angiogenic activity of therapeutic CACs, Toyama et al.8 have treated cultured cells with low-intensity pulsed ultrasound (LIPUS). They go on to demonstrate that LIPUS increases the number of CACs obtained from atherosclerotic patients, making them similar to the yield from normal patients without CAD.7 LIPUS also increases the angiogenic potential of both normal and atherosclerotic patient-derived CACs as indicated by increased angiopoietin 2 secretion, increased eNOS expression, and increased NO concentration. In preclinical work, this pretreatment was successful in increasing the flow and capillary density in a mouse model and may increase neovascularization in future clinical studies.8

The bioeffects of ultrasound have been known for years and this is not the first time LIPUS has been used to treat cells or tissues. LIPUS has been used to promote fracture healing in bone10 and to stimulate healing in a variety of soft tissues.11 Although the mechanism of action has still to be elucidated in detail, LIPUS has been shown to stimulate integrin mechanoreceptors on the cell surface and signal through phosphatidylinositol 3-kinase and Akt to induce differentiation.12 The present study demonstrates increased Akt activation for only 1–3 h, while eNOS was induced for >6 h after LIPUS. It is unclear if and to what degree genes such as eNOS are up-regulated in LIPUS-stimulated CACs over longer time periods, but in hindlimb ischaemia studies, effects are seen 2 weeks after LIPUS treatment, raising the possibility that some of the effects of LIPUS are long lasting. Still, the reduction in eNOS expression over time might be an important limiting factor. The duration of treatment is an important consideration in gene therapy approaches2 and might prove to be important in stimulated

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This editorial refers to ‘Ultrasound stimulation restores impaired neovascularization-related capacities of human circulating angiogenic cells’ by Y. Toyama et al., pp. 448–459, this issue.
cell therapy as well. It would be fascinating to know if LIPUS could be used after injection to boost the expression of angiogenic factors in vivo. Ultrasound has been shown to promote angiogenesis in vivo, and serial treatment more closely resembles standard LIPUS treatment regimes to promote the healing of bone or soft tissue.

It should be noted that while this technique was used to stimulate CACs in this particular study, the technique is able to stimulate a variety of cells to undergo a variety of cellular processes. Serial LIPUS treatment has been used to enhance chondrogenic differentiation in human mesenchymal stem cells in conjunction with appropriate differentiation media. Most recently, LIPUS has been used to increase the proliferation of fresh haematopoietic stem/progenitor cells while enhancing the therapeutic potential as measured by increased erythroid burst-forming units. The present study by Toyama et al. is another illustration of both how and why LIPUS stimulation might enhance the clinical efficacy of cellular therapeutics. Clearly, there will be more applications to come.

References