True Autologous Approach in Cell Therapy
– Using Your Own Serum for Cell Culture –
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Ischemic cardiovascular diseases remain among the most serious health challenges worldwide, despite many breakthroughs in cardiovascular medicine. Recently, approaches using stem or progenitor cells to regenerate these ischemic cardiovascular tissues have emerged as a novel therapeutic option. Currently, various bone marrow (BM)-derived cells including endothelial progenitor cells (EPCs) are being investigated to regenerate or repair ischemic myocardium or limbs depending on the type of disease and the differentiation characteristics of specific stem or progenitor cell populations. The issue has been raised, however regarding the use of animal serum for generation of stem or progenitor cells ex vivo. The use of human serum is a promising alternative for clinical therapy.

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Until recently, vasculogenesis was thought to be restricted to embryonic development, whereas angiogenesis was considered to be responsible for neovascularization in embryos and adults. Now the existence of postnatal vasculogenesis is widely accepted, with the discovery that BM-derived EPCs circulate in peripheral blood (PB), 1 home to and incorporate into foci of neovascularization in adult animals, and increase in number in response to tissue ischemia. Initially, Fk-1 and CD34, shared by angioblasts and hematopoietic cells, were used to isolate putative angioblasts from the mononuclear cell fraction of PB. 1 Meanwhile, EPCs subsequently were isolated from human umbilical cord blood, BM mononuclear cells, and CD34+ or CD133+ hematopoietic stem cells. These cells show various endothelial cell phenotypes during ex vivo culture. In animal models of ischemia, EPCs were shown to incorporate into sites of active neovascularization in ischemic and tumor tissue. Studies have shown the effect of direct repopulation of EPCs into lost endothelial cells in a rat model of myocardial infarction (MI). 2 The systemically administered ex vivo expanded EPCs following MI incorporate into foci of myocardial vasculature and have a favorable impact on preservation of left ventricular function. Other simplified approaches using unselected or selected BM or PB mononuclear cell fractions, 3 have been reported to be successful in improving cardiac function following various models of myocardial and hindlimb ischemia. More recent studies have demonstrated that the therapeutic mechanism of various BM-derived cells are attributed to their capacity to exert paracrine or humoral effects rather than cellular transdifferentiation. 4-5 Regardless of the mechanistic debate, the therapeutic benefits of cell therapy have been reported by most of the experimental animal studies on tissue repair or regeneration.

The promising results from experimental studies prompted the initiation of clinical studies. Pilot clinical studies explored the therapeutic potential of both cultured and freshly isolated EPCs or mononuclear cells. Studies have shown that cells injected into the affected coronary arteries improved global left ventricular function and global microvascular function in post-MI patients. 6 Larger placebo-controlled clinical trials have demonstrated split results showing both favorable or non-significant effects. 7,8 There have been a few studies that addressed the therapeutic effects of uncultured PB or BM mononuclear cells on peripheral vascular obstructive disease or critical limb ischemia. 9-10 Although the number of enrolled patients was small, these studies uniformly demonstrated beneficial effects. At present, studies using either non-cultured or cultured BM cells have demonstrated the feasibility, safety, and bioactivity of these candidate cells in patients with myocardial or critical limb ischemia. Most studies, however, were conducted using uncultured BM or PB mononuclear cells due to the safety concerns associated with culturing cells. One major concern is the use of animal serum. Thus studies using EPCs cultured with a safer method are necessary, because cultured EPCs are one of the originally reported cell types that opened this cell therapy field. As yet no definitive (ie, phase 3) studies have been conducted in any cell-based therapy to date.

The major limitation of using cultured EPCs for clinical use is how EPCs can be expanded on a large scale using a safe culture protocol. Most EPC expansion protocols utilize media containing fetal bovine serum (FBS). Because FBS contains growth factors, attachment factors and vital nutrients, it is one of the most widely used ingredients for cell culture. The use of xenogeneic serum, however, can pose a risk such as disease transmission, harmful immunizing effects or adverse effects due to unknown factors. Potential concerns include viral, prion and zoonose contamination. Anti-FBS antibodies and inflammatory reactions have been detected and these could have immunological adverse events and be a factor influencing therapeutic results. 10 Shumiya et al in this issue of the Journal have evaluated the quality and potential of cultured EPCs using autologous human serum without FBS. 11 That study demonstrated that EPCs could be expanded...
with human serum and that transplanted EPCs augmented blood flow and reduced limb loss and necrosis, suggesting the competence of human serum for clinical use. Other efforts have been made to reduce or exclude the use of FBS. For instance, chemically defined serum-free media could be a possible alternative for large-scale manufacturing, but it has not been fully successful thus far. Another option for the potential FBS substitutes was the use of human blood products such as allogenic human serum from blood and cord blood or platelet derivatives.\textsuperscript{12} Their use, however, is still limited to pre-clinical studies. Currently the most reliable option would be to use autologous serum. \textbf{Table} lists a summary of clinical studies using cultured cells with human serum or plasma.\textsuperscript{13,14} These studies have uniformly demonstrated increased functional improvement and safety, suggesting the utility of human autologous serum for therapeutic use. As demonstrated in experimental hindlimb ischemia in the Shumiya et al study,\textsuperscript{15} a clinical trial with cultured EPCs using autologous serum for patients with peripheral vascular obstructive is desired.

Issues remain regarding the use of autologous serum for expansion and/or differentiation of PB or BM cells. Several studies have demonstrated that serum from patients with heart failure or diabetes has adverse effects on the growth and differentiation characteristics of BM-derived stem or progenitor cells.\textsuperscript{16} Moreover, the study by Shumiya et al. used only PB cells from healthy volunteers.\textsuperscript{11} Similar studies using patients’ PB cells and their own serum may be required to test their potency. Notwithstanding, autologous serum is an ideal choice for culturing cells for cell-based therapy.

\section*{Acknowledgments}

This work was supported in part by National Institutes of Health grants (HL084471, HL97353), a planning grant from GTEC-ACTSI (Georgia Tech-Emory Center for Regeneration and Atlanta Clinical and Translation Science Institute) and a grant (SC4300) from Stem Cell Research Technology Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea. The authors have no conflicting financial interests.

\section*{References}


