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, Flk-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.^{6,7} 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.^{8,9} 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

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Table. Clinical Trials Using Stem/Progenitor Cells Cultured With Autologous Human Serum or Plasma

Disease	Cell source	Serum	Medium	Culture period	Route of cell application	Outcome	Reference
Acute myocardial infarction (20 pts)	BM-MNCs	2% Human plasma	X-vivo 15	1 day in teflon bag	Intracoronary infusion	Reduced infarcted area, Improved cardiac hemodynamics	13
Acute myocardial infarction (54 pts)	BM cells, CPCs	20% Human serum	X-vivo 15 with VEGF	3 days in fibronectin coated dish	Intracoronary infusion	Reduced infarcted area, Improved cardiac hemodynamics	14
Myocardial infarction (20 pts)	BM cells	2% Human plasma	RPMI-1640	1 day in teflon bag	Intracoronary infusion	Reduced infarcted area, Improved cardiac hemodynamics	15

pts, patients; BM, bone marrow; MNC, mononuclear cell; CPC, circulating progenitor cell; VEGF, vascular endothelial growth factor.

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.¹² Their use, however, is still limited to pre-clinical studies. Currently the most reliable option would be to use autologous serum. **Table** lists a summary of clinical studies using cultured cells with human serum or plasma.^{13–15} 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,¹¹ 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.¹⁶ Moreover, the study by Shumiya et al. used only PB cells from healthy volunteers.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.

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References

- Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; **275**: 964–967.
- Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001; **103**: 634–637.
- Shintani S, Murohara T, Ikeda H, Ueno T, Sasaki K, Duan J, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001; **103**: 897–903.
- Cho HJ, Lee N, Lee JY, Choi YJ, Li M, Wecker A, et al. Role of host tissues for sustained humoral effects after endothelial progenitor cell transplantation into the ischemic heart. *J Exp Med* 2007; **204**: 3257–3269.
- Rehman J, Li J, Orschell CM, March KL. Peripheral blood 'endothelial progenitor cells' are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003; **107**: 1164–1169.
- Dimmeler S, Burchfield J, Zeiher AM. Cell-based therapy of myocardial infarction. *Arterioscler Thromb Vasc Biol* 2008; **28**: 208–216.
- Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, et al. Adult bone marrow-derived cells for cardiac repair: A systematic review and meta-analysis. *Arch Intern Med* 2007; **167**: 989–997.
- Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Therapeutic angiogenesis for patients with limb ischemia by autologous transplantation of bone-marrow cells: A pilot study and a randomised controlled trial. *Lancet* 2002; **360**: 427–435.
- Kajiguchi M, Kondo T, Izawa H, Kobayashi M, Yamamoto K, Shintani S, et al. Safety and efficacy of autologous progenitor cell transplantation for therapeutic angiogenesis in patients with critical limb ischemia. *Circ J* 2007; **71**: 196–201.
- Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci USA* 2002; **99**: 8932–8937.
- Shumiya T, Shibata R, Shimizu Y, Ishii M, Kubota R, Shintani S, et al. Evidence for the therapeutic potential of ex vivo expanded human endothelial progenitor cells using autologous serum. *Circ J* 2010; **74**: 1006–1013.
- Lange C, Cakiroglu F, Spiess AN, Cappallo-Obermann H, Dierlamm J, Zander AR. Accelerated and safe expansion of human mesenchymal stromal cells in animal serum-free medium for transplantation and regenerative medicine. *J Cell Physiol* 2007; **213**: 18–26.
- Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: Final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol* 2004; **44**: 1690–1699.
- Fernandez-Aviles F, San Roman JA, Garcia-Frade J, Fernandez ME, Penarrubia MJ, de la Fuente L, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. *Circ Res* 2004; **95**: 742–748.
- Cebotari S, Lichtenberg A, Tudorache I, Hilfiker A, Mertsching H, Leyh R, et al. Clinical application of tissue engineered human heart valves using autologous progenitor cells. *Circulation* 2006; **114** (1 Suppl): I132–I137.
- Chen YH, Lin SJ, Lin FY, Wu TC, Tsao CR, Huang PH, et al. High glucose impairs early and late endothelial progenitor cells by modifying nitric oxide-related but not oxidative stress-mediated mechanisms. *Diabetes* 2007; **56**: 1559–1568.