A Novel Method for High Efficiency Vascular Progenitor Recovery

Bryan W. Tillman[1,2], Saami K. Yazdani[1], Anthony Atala[1], Randolph L. Geary[2], Matthew Corriere[2], and James J. Yoo[1]

[1] Wake Forest Institute for Regenerative Medicine and [2] Department of Surgery, Section on Vascular and Endovascular Surgery

Introduction

Autologous CD133 positive progenitor cells have been differentiated into a variety of cell types with clinical utility. Cells expressing CD133 constitute the progenitors of diverse cell types such as hepatocytes, neurons, vascular endothelium, and smooth muscle cells. These attributes have led to the use of CD133 positive cells in clinical trials for a variety of applications including cardiac, hepatic, and muscle regeneration as well as in bone marrow transplantion. In addition, cells expressing CD133 have been used as the progenitors for various bioengineered tissues. However, CD133 positive cells are infrequent in isolated peripheral blood, ranging from about 0.01 to 0.0001% of leukocytes. The low numbers of circulating CD133 positive endothelial progenitor cells with limited cell passage remain a major obstacle for potential clinical applications. In addition, the volume of blood that can be safely removed from a living donor is limited and this, in turn, minimizes the yield of prospective progenitor cells for expansion. Herein, we describe an approach using an Extracorporeal Cell Affinity (ECA) column to recover CD133 positive progenitor cells from nearly the entire peripheral blood volume of a living donor while minimizing losses of other hematopoetic cell types.

Methods

Affinity matrix was prepared by conjugation of purified anti-CD133 antibody to sepharose beads. Columns were assembled with beads that are evenly distributed amongst three tiers. The ECA columns consisted of sepharose matrix with antibody based affinity towards the progenitor antigen CD133 retained by mesh filters (Figure 1). The extracorporeal circuit consisted of an arterial cannula carrying blood to the column and the blood was then returned to the donor by a venous cannula to complete the circuit. Sheep blood was cycled through the column for a volume of 1800 mL using surgical cannulation of superficial vessels. As control, blood specimens were alternately processed using a conventional buffy leukocyte protocol. Given the expected turbulent environment of a device cycling high volumes of blood and the theoretical potential for allergic reaction or disturbed hematologic parameters, perioperative hematologic and physiologic parameters were assessed in column cycled animals before and after cycling of the ECA column as well as in control animals (n = 8). Recovered cells were assessed by immunohistochemistry (IHC) for expression of CD133. Colony counts were compared between normalized column purified cells versus traditional buffy coat specimens at 9 days after plating. The resulting cells were validated for expression of endothelial markers by IHC and the growth curves were compared between the column and buffy coat methods.

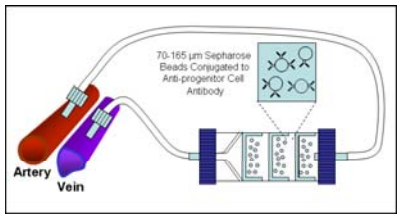

Figure 1: Schematic diagram of extracorporeal cell affinity (ECA) column. An arterial cannula carries peripheral blood to the column, which contains three tiers of mesh filters retaining antibody-coated sepharose beads (70 – 165 mm diameter, inset). CD133+ cells are retained in the column while unbound cells and plasma are returned to the donor by a venous cannula.

Results

A postoperative elevation of neutrophils and eosinophils was small but statistically significant from the control animals. No significant depletion of other cell types was noted (Table 1A). Physiologic parameters were not significantly different between column cycled versus control operated control animals ($p > 0.05$ for all variables) (Table 1B). Column purified cell suspensions demonstrated enrichment of CD133 cells was observed compared to those in buffy leukocyte preparations (Fig.1). With regards to colony forming capacity, when cultured for four weeks, the average yield of buffy leukocytes was only 0.72 colonies from each 10 mL of peripheral blood (n=18). In contrast, a single run of the ECA column generates over 600 fold more endothelial colonies than a 10 mL blood specimen buffy coat. When outgrowth cells were compared between the two methods, both exhibited similar staining profiles for endothelial markers (Fig. 4). To ascertain the time advantage for this column based approach in reaching a target endpoint of 10 million cells, an isolated blood specimen, 10 milliliters (mL) processed as buffy leukocytes, was compared to cells recovered from a single column cycle. As illustrated in Table 2, the increased starting number of progenitor cells from the ECA column conferred an expected expansion advantage, attaining the endpoint at a lower passage nearly three times earlier ($p < 0.0001$) than the buffy leukocyte derived endothelial cells.

A.

Physiologic Parameter	Sham Control Mean (n=8)	ECA Column Mean (n=8)	p-value
Heart Rate	95.74	104.63	0.489
Systolic BP	98.02	106.62	0.982
Diastolic BP	64.34	70.92	1.000
End-Tidal CO2	88.70	93.90	1.000
O2 Saturations	45.41	42.65	0.863

B.

	Sham Control (n=8)			ECA Column (n=8)			
Cell Type	Pre	Post (24 h)	Change	Pre	Post (24 h)	Change	p-value
WBC	5.25	6.73	1.48	5.12	8.15	3.03	0.130
Neutrophil	2.04	2.81	0.77	1.75	4.05	2.30	0.028
Eosinophil	0.18	0.19	0.01	0.16	0.37	0.21	0.065
Hematocrit	31.56	33.31	1.75	33.43	44.14	10.71	0.010
Platelets	443.13	355.38	-87.75	496.75	526.38	29.63	0.161

Table 1: (A) Comparison of intraoperative physiologic parameters between control and ECA column cycled sheep. BP: blood pressure, CO2: carbon dioxide, O2: oxygen (B) Comparison of cell counts. Statistical significance was assessed comparing changes between pre and post 24 hours after column cycling or sham surgery (n=8 for each group). Pre values were collected intraoperatively, immediately prior to column cycling. Units are cells/mm³ except hematocrit which is expressed as percentage.

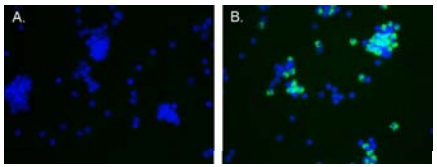

Figure 2: Comparison of CD133 expression on ECA column eluted cells compared to buffy coat leukocytes. Buffy coat leukocytes (A) or Column eluted cells (B) were dual stained with the nuclear stain DAPI (blue) and anti-CD133 FITC (green) and examined by fluorescent microscopy at 400X magnification as shown.

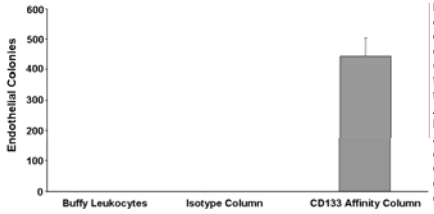

Figure 3: Endothelial colony assay of column eluted cells versus controls. Cells recovered after cycling 1800 mL of blood through the CD133 ECA column were plated on fibronectin coated 24-well plates at twenty-five thousand cells per well. An equivalent density of buffy coat leukocytes and cells recovered from an IgG isotype ECA column and observed for endothelial colony outgrowths (n=4 for each of column groups and n=8 for buffy leukocyte cells).

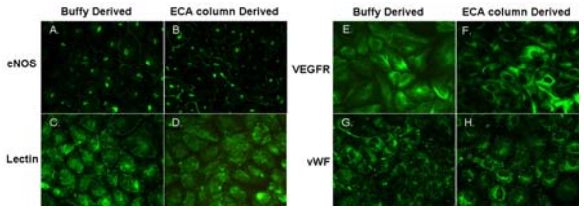

Figure 4: Comparison of immunohistochemical (IHC) staining of endothelial markers between buffy leukocyte derived endothelial cells and column derived endothelial cells. IHC staining for eNOS (A and B), Lectin (C and D), VEGFR (E and F), and vWF (G and H) are shown for buffy leukocyte derived cells (A, C, E, and G) and CD133 ECA column eluant derived endothelial cells (B, D, F, and H). Representative images are shown.

Endothelial Cell Source	Median time to 10 million cells	95% Confidence Interval
Buffy Leukocyte (n=18)	35 days	(34.0 – ∞)
ECA Column (n=4)	12.5 days	(12.0 – 14.0)

Table 2: Assessment of median growth time for buffy leukocyte derived versus column derived endothelial cells. When expanded at an equivalent cell density with each passage, column derived endothelial colonies achieve a target endpoint of 10 million cells significantly sooner that buffy leukocyte derived colonies isolated from peripheral blood specimens. Median number of days to achieve the 10 million cell target along with the 95% confidence interval for survival analysis is shown. ($p < 0.0001$)

Conclusions

Reports of decreased numbers of vascular progenitors in smokers, diabetics, and patients with cardiovascular disease suggest a more efficient means to recover endothelial progenitor cells will be required for clinical application.Our results suggest that ECA technology may facilitate the generation of large numbers of progenitor derived cells for clinical therapies and reduce the time required to attain clinically relevant cell numbers. The device is well tolerated during *in vivo* application with no effects on cell counts or physiological parameters. *Ex vivo* trials of this system in humans are currently underway. Ultimately, this approach may facilitate cell based therapies that use CD133 and other peripheral progenitors.

References

Ingram, D.A., N.M. Caplice, and M.C. Yoder, *Unresolved questions, changing definitions, and novel paradigms for defining endothelial progenitor cells.* Blood, 2005. **106**(5): p. 1525-31.

Peichev, M., et al., *Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors.* Blood, 2000. **95**(3): p. 952-8.

Kawamoto, A. and T. Asahara, *Role of progenitor endothelial cells in cardiovascular disease and upcoming therapies.* Catheter Cardiovasc Interv, 2007. **70**(4): p. 477-84.

Acknowledgements

We thank Cindy Andrews and Mandy Lockard for technical assistance. This work was supported, in part, by the Department of Defense.

Wake Forest Institute *for* REGENERATIVE MEDICINE