Buoyant targeted microbubble technology provides efficient and superior quality of hematopoietic stem cells isolated from umbilical cord blood

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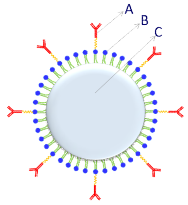
Introduction

Various separation methods to isolate and enrich hematopoietic stem/progenitor cells (HSPC) have been developed. However, there is still growing interest in clinical trial settings to purify CD34+ cells for ex vivo HSPC expansion and for gene therapy. We have recently developed BUBLES (Buoyancy Enabled Separation), an innovative cell sorting technology using the lipid shell microbubble that is self-molding to external forces. In conjunction with a gas core, it is a very gentle material for cell isolation. This patented technology has the potential to overcome some key hurdles of cell therapy in humans, including targeted multi-parametric cell isolation in bulk for clinical applications. In this study, we aimed to demonstrate that the BUBLES technology is able to efficiently enrich CD34+ HSPC from umbilical cord blood (UCB) and preserve the unique characteristics of stem cells by both in vitro cell assays and in vivo animal study.

BUBLES for Cell Selection



	Materials	Functions
A	Ligands (e.g. antibodies)	Target specificity
B	Lipids	Mimic cell membrane and maintain the structure
C	Gas	Floating Cushioning cells Releasing cells

Figure 1. Structure and function of microbubbles.

Lipid microbubbles were used to positively select cells. A diagram showing the typical structure of immuno-MBs, which was composed of 3 key parts:

A. Targeting molecules used to capture targeted cells;
B. Outer lipid shell used to mimic cell membrane structure and maintain the structure;
C. Inner gas core used to provide buoyant force for separation, to enable elastic deformation for cushioning cells from damage, and to release cells by popping microbubbles.

Cell Colony Forming Assay

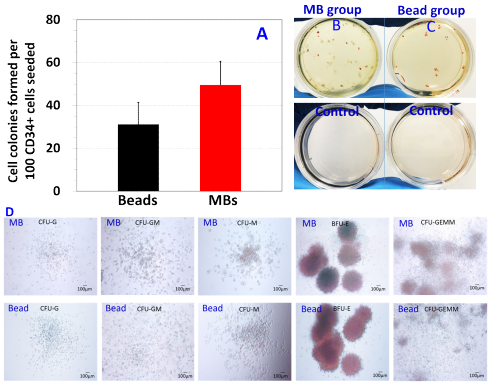
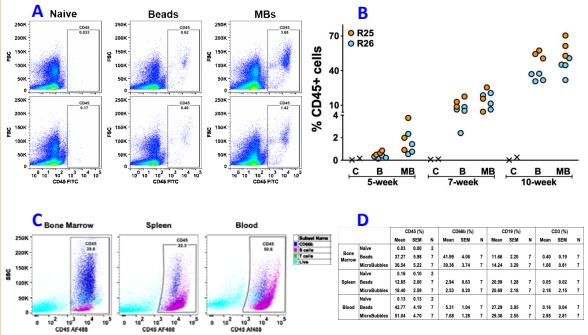


Figure 3. Cell colony forming assay. (A). Cell colonies formed at 2 week per 100 CD34+ HSPC cells seeded (n=4); Representative cell colony formation on a 35-mm petri dish of MB group (B) and Bead group (C); Representative microscopic photos showing various cell colony morphologies of MB group (upper row) and Bead group (lower row).

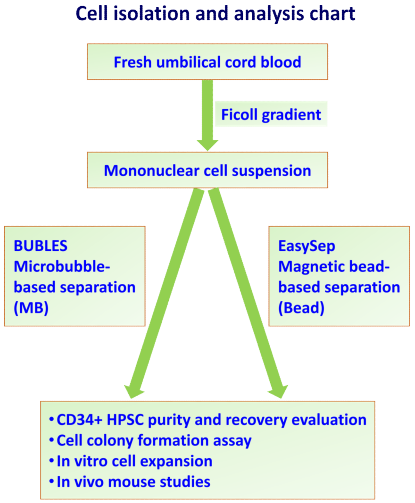
In Vivo Animal Study



		CD45 (%)			CD34% (%)			CD19 (%)			CD3 (%)		
		Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Bone Marrow	Naive	0.03	0.00	2									
	Beads	37.27	6.86	7	41.99	4.08	7	11.66	3.20	7	0.40	0.19	7
	MicroBubbles	36.54	5.23	7	39.36	3.74	7	14.24	3.29	7	1.66	0.81	7
Spleen	Naive	0.16	0.10	2									
	Beads	12.85	2.80	7	2.54	0.63	7	20.39	1.28	7	0.85	0.62	7
	MicroBubbles	10.40	2.59	7	2.03	0.39	7	20.68	2.18	7	2.10	2.15	7
Blood	Naive	0.13	0.13	2									
	Beads	42.27	4.79	7	5.31	1.04	7	27.28	3.85	7	0.16	0.04	7
	MicroBubbles	51.84	4.70	7	7.68	1.28	7	29.36	2.95	7	2.95	2.81	7

Figure 5. Mouse engraftment results of isolated CD34+ HSPC cells from 2 donors. (A). Representative FACS plots showing robust engraftment as early as 5 weeks post-TP; (B). Enhanced or comparable engraftment levels by MB vs. bead-method over the 10-week transplantation period as seen in peripheral blood; (C). FACS plots showing human CD45+ and differentiated cells in hematopoietic tissues from mice after 10 weeks; (D). Table summary for human CD45+ cells and differentiation in tissues from mice.

Method



Cells isolated by the BUBBLES technology were released by popping the microbubbles under air pressure at 3 atm.

Cell Purity and Recovery

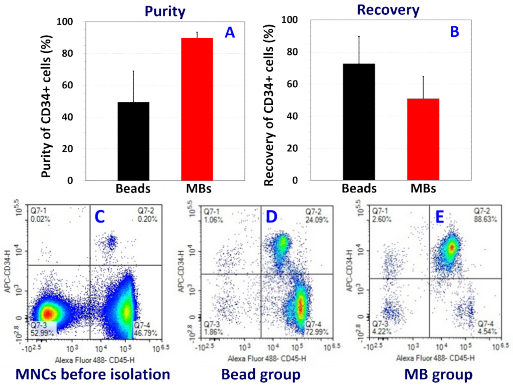


Figure 2. CD34+ HSPC cells isolated from fresh human umbilical cord blood by using BUBLES technology (MB group) and magnetic bead technology (Bead group). (A). Cell purity (n=6); (B). Cell recovery (n=6); Representative flow cytometry dot plots showing CD34+/CD45+ cell population of MNCs before isolation (C), Bead group (D) and MB group (E), respectively.

In Vitro Cell Expansion

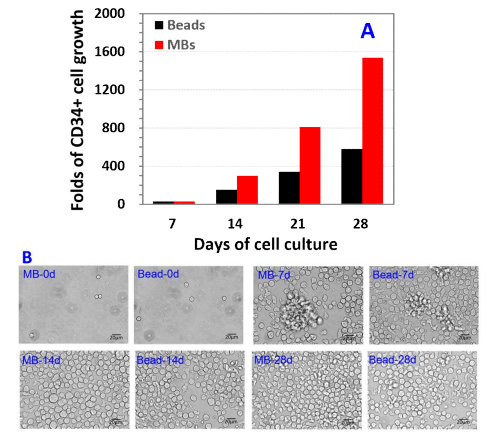


Figure 4. Cell expansion results of isolated CD34+ HSPC cells from 2 donors. (A). Fold changes of isolated HSPC cells cultured in the expansion-defined media; (B). Representative microscopic photos showing the morphology of cultured HSPC cells.

Conclusions

- The average purity of CD34+ HSPC cells isolated from human UCB by microbubble-based BUBLES technology was higher than that by the EasySep bead method.
- Comparable recovery rate of isolated human CD34+ HSPC cells was observed using both methods.
- The average colony-forming units per 100 CD34+ cells were higher in the BUBLES group versus the Bead group.
- A higher *in vitro* CD34+ HSPC cell expansion was observed from the BUBLES technology compared to the EasySep bead method.
- Engraftment in the peripheral blood of transplanted mice with BUBLES-isolated CD34+ cells was detected at 5-week post-transplantation (1.7%±0.4%) whereas no engraftment was observed in those with EasySep. Normal B and T cell differentiation was observed in both groups of transplanted mice after 8 weeks.
- Based on both *in vitro* and *in vivo* results, we have demonstrated that BUBLES technology effectively isolates CD34+ HSPC from UCB and preserves the stemness quality.

ACKNOWLEDGEMENT

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