HHS Public Access

Author manuscript

Stem Cell Res. Author manuscript; available in PMC 2021 November 29.

Published in final edited form as:

Stem Cell Res. 2021 October ; 56: 102531. doi:10.1016/j.scr.2021.102531.

Polyvinyl alcohol hydrolysis rate and molecular weight influence human and murine HSC activity ex vivo

Kazuhiro Sudo[a,*], **Satoshi Yamazaki**[b,c,*], **Adam C. Wilkinson**[d], **Hiromitsu Nakauchi**[b,e], **Yukio Nakamura**[a]

[a]Cell Engineering Division, BioResource Research Center, RIKEN, Tsukuba, Japan

[b]Division of Stem Cell Therapy, Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

[c]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan

[d]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK

[e]Institute for Stem Cell Biology and Regenerative Medicine and Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA

Abstract

Ex vivo expansion of hematopoietic stem cells (HSCs) is one of the most promising strategies to increase the availability of transplantable HSCs and improve bone marrow transplantation outcomes. We recently demonstrated that mouse HSCs could be efficiently expanded in polyvinyl alcohol (PVA)-containing culture medium using only recombinant stem cell factor and thrombopoietin cytokines. However, the behavior of human HSCs in these simple PVA-based media was not fully elucidated. In this study, we analyzed the compatibility of PVA of different hydrolysis rates (HR) and molecular weights (MW) to support functional human and mouse HSCs ex vivo. Human and mouse HSCs proliferated more frequently in media containing PVA with lower HR than with higher HR, but both PVA types supported HSC multilineage reconstitution potential. Importantly, human HSCs cultured in PVA-containing media engrafted not only in irradiated recipients but also in non-irradiated recipients. Our results demonstrate that human

[*]Corresponding authors at: Cell Engineering Division, RIKEN BioResource Research Center, 3-1-1, Koyadai, Tsukuba, Ibaraki 305-0074, Japan (K. Sudo). Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan. (S. Yamazaki). kazuhiro.sudo@riken.jp (K. Sudo), y-sato4@md.tsukuba.ac.jp (S. Yamazaki).

Authorship contributions

K.S designed the research, performed the experiments, analyzed data and wrote manuscripts. S.Y conceptualized the research, performed the experiments, analyzed data. A.C.W. edited the manuscript. H.N and Y.N supervised experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Disclosure of conflicts of interest

H.N is a co-founder and shareholder of ReproCELL. Inc.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102531.

HSCs can be maintained ex vivo using PVA-based culture systems and suggest approaches for future optimization of human HSC expansion.

Keywords

Hematopoietic stem cell; Ex vivo expansion; Polyvinyl alcohol; Transplantation; Non-conditioned recipient

1. Introduction

Hematopoietic stem cell (HSC) transplantation is now a standard therapy for the treatment of various hematological disorders such as leukemias and inherited anemias (Passweg et al., 2016). The bone marrow (BM) has long been the major source of HSCs, but in recent years use of umbilical cord blood (UCB) HSCs has become more frequent. However, the number of HSCs in a single UCB is often low (Notta et al., 2011; Wilkinson et al., 2020a). This represents a major limitation to the wider application of this otherwise accessible HSC source for transplantation. The generation of ex vivo culture systems that efficiently expand human HSCs are therefore essential for the greater clinical use of UCB HSCs. A number of protocols to expand human HSCs are being trialed (Cohen et al., 2020; Dahlberg et al., 2011; Delaney et al., 2010; Papa et al., 2020). It has long been thought that serum albumin is the key factor to maintain and expand HSCs within ex vivo cultures (Ieyasu et al., 2017). However, we previously showed that bovine serum albumin (BSA) can be a major source of biological variability in HSC cultures (Ieyasu et al., 2017) and recently discovered that serum albumin could be entirely replaced with polyvinyl alcohol (PVA) (Wilkinson et al., 2019). PVA is a large class of synthetic resins formed by the hydrolysis of polyvinyl acetate and can vary in both molecular weight (MW) and hydrolysis rate (HR). Mouse HSCs could be cultured for more than one month in PVA-containing culture conditions while maintaining their functional properties (Wilkinson et al., 2019). Given that PVA is a Good Manufacturing Practice (GMP) compatible reagent, such an approach is potentially directly translatable to human HSC expansion for clinical applications. Here, we detail how PVA MW and HR influence the ex vivo culture of human UCB $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) and mouse BM HSCs. Our results highlight how the selection of the appropriate PVA type is key for optimizing HSC expansion ex vivo.

2. Materials and methods

2.1. Mice

C57BL/6-CD45.2 and C57BL/6N-CD45.1 (PepboyJ) mice were purchased from Japan SLC, Sankyo-Laboratory Service, The Jackson Laboratory or bred in-house. NOG (NOD.Cg-$Prkdc^{scid}$ IL-2rγ^{null}/SzJ) mice were purchased from In Vivo Science. All mice were housed in specific-pathogen-free condition. All animal protocols were approved by the Animal Care and Use Committee of the Institute of Medical Science University of Tokyo and/or the Animal Care and Use Committee of RIKEN Tsukuba Branch.

2.2. Mouse and human HSCs

Mouse HSCs were obtained from mouse BM as previously described (Wilkinson et al., 2019; Wilkinson et al., 2020b). Anonymized human cord blood $CD34^+$ cells were purchased from Cell Engineering Division, BioResource Research Center, RIKEN.

2.3. PVA

All PVA were purchased from SIGMA (Cat# 348406, 363146, 363103, P8136, 363170, 363081, 363065, 363138). Their molecular weights and hydrolysis rates were summarized in Fig. 1a. To obtain 10% (w/v) PVA solution, all PVAs were respectively dissolved in water by autoclaving.

2.4. Mouse HSC culture

Mouse HSC cultures were performed as previously described (Wilkinson et al., 2020b). Briefly, mouse $CD34^-$KSL HSCs were seeded in fibronectin (FN)-coated 96-well plates and cultured in the medium consisted with F12 medium (Life Technologies), 1% ITS-X (Life Technologies), 1% penicillin/streptomycin/glutamine (P/S/G; Life Technologies), 10 mM HEPES (Life Technologies), 0.1% PVA (348406, 363146, 363103, P8136, or 363081) or 0.1% recombinant human serum albumin (rHSA; Albumin Biosciences), 10 ng/ml recombinant mouse stem cell factor (mSCF; Peprotech) and 100 ng/ml recombinant mouse thrombopoietin (mTPO; Peprotech). Cytokines were dissolved in PVA-or rHSA-containing culture medium. Cell cultures were performed at 37 °C with 5% CO_2 and 20% O_2.

2.5. Competitive transplantation assay

Cultured HSCs derived from C57BL/6N-CD45.1 mice were transplanted with 1×10^6 whole BM cells obtained from C57BL/6N-CD45.1/ CD45.2 (F1) mice into lethally irradiated (9.5 Gy) C57BL/6N-CD45.2 mice. Peripheral blood (PB) was collected every 4 weeks after transplantation. PB mononuclear cells were stained with the combination of 7 antibodies; anti-mouse CD45.1-PE/Cy7 (Biolegend), anti-mouse CD45.2-BV421 (Biolegend), anti-mouse CD11b-PE (eBiosciences), anti-mouse Ly-6G/Ly-6C-PE (eBiosciences), anti-mouse CD45RA-APC/ eFluor780 (eBiosciences), anti-mouse CD4-APC (eBiosciences), and anti-mouse CD8-APC (eBiosciences). Cells were analyzed by FACS Aria Special Order Research Product (SORP Aria). For secondary BM transplantation, total BM cells were obtained from the primary mice and 1×10^6 cells were transferred into lethally irradiated C57BL/6N-CD45.2 mice. The chimerism was analyzed as above.

2.6. Human $CD34^+$ cell culture and transplantation

A single donor derived CB $CD34^+$ cells were used for each experiment. Frozen $CD34^+$ cells were thawed and seeded into Ultra low attachment 96-well U-bottomed tissue culture plates (Corning) in PVA-containing medium consisted with IMDM (Life Technologies), 0.1% PVA (348406, 363146, 363103, P8136, 363170, 363081, 363065, 363138) or 0.1% rHSA, 1% ITS-X (Wako), 1% GlutaMax (Life Technologies), 10 mM HEPES (Life Technologies), 20 ng/ml recombinant human SCF, and 50 ng/ml recombinant human TPO. Cell cultures were performed at 37 °C with 5% CO_2 and 20% O_2. Medium was completely changed every 3–4 days. At day seven of culture, cells were used for cell count (using a Vi-CELL

XR; Beckman Coulter), flow cytometric analysis or transplantation. For flow cytometric analysis, cells were stained with anti-human CD45RA-FITC (BioLegend), anti-human CD90-PE (BioLegend), anti-human CD34-APC (BioLegend), anti-human CD38-PE/Cy7 (BD) and Brilliant Violet 421 (BV421) conjugated anti-human CD49f (BD). Cells were analyzed by FACS Verse. For transplantation, 5×10^4 cells were cultured for 7 days in PVA-containing medium and intravenously transplanted into sub-lethally (2 Gy) irradiated NOG mice. The chimerism of human $CD45^+$ cells in the PB and BM cells were analyzed at 16 weeks post-transplantation by flow cytometry following staining with anti-mouse CD45-PE/Cy7 (eBioscience), anti-human CD45-FITC (BD), anti-human CD19-PE (BD), anti-human CD33-APC (BD), anti-human CD3-APC/Cy7 (BioLegend), and anti-human CD34-BV421 (BD).

2.7. Human HSPCs sorting and culture

Frozen $CD34^+$ cells were thawed and washed with IMDM. The cells were stained with anti-human CD45RA-FITC, CD90-PE, CD34-APC, CD38-PE/Cy7 and CD49f-BV421. Fifty cells from both cell populations, $CD34^+CD38^-CD90^+CD45RA^-CD49f^+$ cells or $CD34^+CD38^-CD90^+CD45$ RA^-CD49f^- cells, were sorted using SORP Aria by direct sorting into Ultra Low Cluster U-bottomed 96-well plates filled with media described above. Sorted cells were cultured without medium change for 7 days in and cell numbers were counted under the microscopy at day 7.

2.8. Non-conditioned transplantation

A single donor derived CB $CD34^+$cells were used for one experiment. 1×10^4 $CD34^+$ cells were injected into 7–9 weeks old non-irradiated female NOG mice, or were cultured in PVA-C or -H-containing medium for 7 days and then cells expanded from 5×10^4 $CD34^+$ were divided and transplanted into five non-irradiated NOG mouse. The chimerism of human $CD45^+$ cells in the PB and/or BM cells were analyzed at 12–16 weeks post-transplantation as described above.

2.9. Statistical analysis

Tukey-Kramer tests were performed following one-way ANOVA tests if significant differences were detected in one-way ANOVA. In case of the comparison between two groups, Welch's *t*-test were performed to determine the significance. All statistical analysis were performed by using BellCurve for Excel (Social Survey Rearch Information Co., Ltd).

3. Results and discussion

We initially screened 8 different types of PVA (Fig. 1a) in mouse HSC cultures. Highly purified mouse $CD34^{-/lo}Kit^+Sca1^+Lin^-$ BM HSCs were cultured for 7 days in media containing PVA (PVA-A to PVA-H) or rHSA. Consistent with our previous study (Wilkinson et al., 2019), cells cultured in PVA with 87–89% HR (lower HR; LHR-PVA) divided much more frequently than cells in PVA with > 98% HR (high HR; HHR-PVA) (Fig. 1a). In contrast to HR, PVA MW minimally altered cell proliferation of mouse HSCs. The water solubility of each PVA varied and 10% aqueous solutions of PVA-B, -D, and -E were very viscous and hard to measure accurately. Based on the above results and the difficulty

of dissolving PVA-B, -D, and -E, we selected two HHR-PVA (PVA-A and -C) and three LHR-PVA (PVA-F, -G, and -H) types for analysis in competitive transplantation assays.

Transplantation of 7-day cultured mouse HSCs showed that PVA type altered functional potential (Fig. 1b–c). PVA-A cultures poorly reconstituted hematopoiesis in the recipient mice, suggesting PVA-A only weakly supported HSCs ex vivo. By contrast, PVA-C, -F, and -H supported high multilineage chimerism in recipients (Fig. 1b–c). Interestingly, the cells cultured in PVA-C and PVA-H containing media displayed comparable hematopoietic reconstitution in recipient mice, despite PVA-C supporting significantly less cellular proliferation during the 7-day in culture (Fig. 1a–c). Secondary transplantation assays further revealed that cells cultured in both PVA-F and PVA-H retained strong HSC activity in primary recipients. On the other hand, the chimerism of the PVA-C culture gradually reduced over time (Fig. 1d). These results suggested that PVA-F and PVA-H supported proliferation and expansion of mouse long-term (LT)-HSCs more efficiently than PVA-C, and that PVA-F and PVA-H did not compromise HSC activity after active cell division.

Having characterized how mouse HSCs responded to different PVA types, we next cultured human UBC $CD34^+$ HSPCs in PVA-based media. Similar to mouse HSCs, more frequent human cell proliferation was observed after 14-day culture with LHR-PVA compared to the culture with HHR-PVA except for PVA-C (Fig. 2a). However, the number of cells in HHR-PVA and LHR-PVA were not so significantly different at Day-7 (Fig. S2). To evaluate the reactivity of HSCs and HPCs to PVA, $CD34^+$ cells were fractionated and cell subsets cultured in PVA-containing media. When phenotypically purified human HSCs ($CD34^+CD38^-CD90^+CD45RA^-CD49f^+$ cells: $CD34^+49f^+$ cells) were cultured in PVA-containing media for 7 days, minimal proliferation was observed in HHR-PVA, while more frequent proliferation was observed in the LHR-PVA (Fig. 2b). On the other hand, phenotypic multipotent progenitor cells ($CD34^+CD38^-CD90^+CD45RA^-CD49f^-$: $CD34^+49f^-$ cells) slowly proliferated in PVA-A, C and D and failed to proliferate in PVA-B (Fig. 2b). Although $CD34^+49f^-$ cells tended to proliferate more frequently than $CD34^+49f^+$ cells, only PVA-H displayed a significant difference. Flow cytometric analysis confirmed that after 7–14-day cultures, phenotypic $CD34^+CD38^-$ HSPCs were maintained in all PVA types (Fig. S1a-b). Additionally, this analysis revealed that LHR-PVA cultures contained more $CD90^-49f^+$ cell population than cells in HHR-PVA (Fig. S3).

The engraftment potential of human $CD34^+$ cells cultured in PVA-A, -C, -F, and -H were next assessed by transplantation into irradiated immunodeficient NOD.Cg-Prkdc^{scid} IL-2rγ^{null}/SzJ (NOG) mice. PVA-G was not included in this analysis because mouse HSCs cultured in PVA-G displayed poor long-term hematopoietic reconstitution capacity. $CD34^+$ cell samples obtained from two distinct donors were individually evaluated in these transplantation experiments. In both of experiment, the cells cultured in all PVAs could reconstitute human hematopoiesis in the recipient mice, although the percentages of chimerism varied (Fig. 3a). Notably, PVA cultured HSPCs generally displayed higher BM engrafted than albumin-based cultures. Additionally, PVA-A, -C, -F, and -H supported multilineage reconstitution of B-, T-, and myeloid lineage (Fig. 3b).

We previously reported that mouse HSCs expanded in PVA could home to the BM and produce B, T, and myeloid cells even when the recipient mice did not receive any pre-conditioning such as irradiation (Wilkinson et al., 2019). It has been reported that non-cultured UCB $CD34^+$ cells can also home to the non-irradiated immunodeficient recipient mouse BM (Brehm et al., 2012; McIntosh et al., 2015). The BM microenvironment in non-irradiated recipient mice should be much competitive than that in irradiated mice for cultured human $CD34^+$ cells to home and engraft. To test whether PVA-cultured human cells could achieve the same results, we transplanted 7-day PVA-C and -H cultured human hematopoietic cells into non-conditioned NOG mice (cultures initiated with 1×10^4 $CD34^+$ cells). Although the PVA-H cultured cell chimerism was significantly lower in one experiment (Fig. S3), human hematopoiesis was detected in non-conditioned recipient PB and BM at 16-week after transplantation for all groups, confirming that PVA-cultured human HSPCs retained the capacity to engraft in non-conditioned recipients. (Fig. 3c, Fig. S4).

Based on these results, we conclude that these PVA-based culture conditions can maintain human HSCs capable of engraftment in irradiated and non-irradiated recipients. However, further optimization of human HSC culture conditions is warranted. For example, the addition of UM171, reported to support HSC expansion ex vivo (Fares et al., 2014), may promote expansion of human HSCs in these PVA culture conditions. With future improvements, we believe that this proof-of-concept for non-conditioned transplantation of PVA-culture expanded human $CD34^+$ cells may become feasible for patients.

We were interested to observe differences in the reactivity of human and mouse HSCs to PVA-A. As shown in Fig. 1a and Fig. 3, PVA-A support long-term repopulating ability of human HSC but not of mouse HSCs. However, the difference in the reactivity to PVAs between mouse and human HSCs might be due to the purity of HSC fraction tested. Human $CD34^+$ cells are a much more heterogeneous population of stem and progenitor cells than the highly-purified $CD34^{-/lo}Kit^+Sca1^+Lin^-$ mouse HSCs we used in the mouse HSC assays. Culture and transplantation of a purified human HSC fraction, such as $CD34^+49f^+$ cell fraction, should help to answer this question in future.

Our results highlight the importance of the selecting the most supportive PVA for ex vivo expansion cultures. From the 8 PVA types tested, PVA-H was most supportive for mouse HSC expansion. Our data also suggest that PVA type may differentially support LT-HSCs, ST-HSCs, and other cell types. For example, the high reconstitution in primary recipients but low reconstitution in secondary recipients from PVA-G cultures suggests it promoted ST-HSC generation. Additionally, despite large differences in cellular proliferation in PVA-C and PVA-H, similar levels of long-term reconstitution were observed, suggesting PVA-H may also support expansion of non-engrafting cells. However, we cannot discount these similar reconstitution levels were due to saturation in the recipient mice.

We hope that future molecular studies comparing HSCs within these different PVA cultures will help to decipher the function of PVA in supporting HSCs. While we have found that PVA stabilizes cytokines in media similar to rHSA (Nishimura et al., 2019), we believe PVA likely plays additional roles in supporting HSCs. PVA may work as the carrier of other substances such as trace elements or amino acids that are necessary for cell survival,

proliferation, and maintenance of HSCs. The difference of MW and HR possibly give different performance of PVA as a carrier. We hope that if the mechanisms of how PVA functions in HSC culture can be uncovered, one of the most uncontrollable somatic stem cells, the HSC, may finally become “controllable”.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all members in Cell Engineering Division, BRC, RIKEN for their help. S.Y is funded by JSPS KAKENHI Grant-in-Aid for Scientific Research (JP18H05095; JP17H05086) and the Japan Agency for Medical Research and Development (JP18bm0404025). A.C.W. was funded by a Leukemia and Lymphoma Special Fellow (3385-19), the NIH (K99HL150218), and the Kay Kendall Leukaemia Fund.

References

Brehm MA, Racki WJ, Leif J, Burzenski L, Hosur V, Wetmore A, Gott B, Herlihy M, Ignotz R, Dunn R, Shultz LD, Greiner DL, 2012. Engraftment of human HSCs in nonirradiated newborn NOD-scid IL2rgamma null mice is enhanced by transgenic expression of membrane-bound human SCF. Blood 119, 2778–2788. 10.1182/blood-2011-05-353243. [PubMed: 22246028]

Cohen S, Roy J, Lachance S, Delisle J-S, Marinier A, Busque L, Roy D-C, Barabé F, Ahmad I, Bambace N, Bernard L, Kiss T, Bouchard P, Caudrelier P, Landais S, Larochelle F, Chagraoui J, Lehnertz B, Corneau S, Tomellini E, van Kampen JJA, Cornelissen JJ, Dumont-Lagacé M, Tanguay M, Li Q.i., Lemieux S, Zandstra PW, Sauvageau G, 2020. Hematopoietic stem cell transplantation using single UM171-expanded cord blood: a single-arm, phase 1–2 safety and feasibility study. Lancet Haematol. 7 (2), e134–e145. 10.1016/S2352-3026(19)30202-9. [PubMed: 31704264]

Dahlberg A, Delaney C, Bernstein ID, 2011. Ex vivo expansion of human hematopoietic stem and progenitor cells. Blood 117, 6083–6090. 10.1182/blood-2011-01-283606. [PubMed: 21436068]

Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID, 2010. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat. Med 16 (2), 232–236. ht10.1038/nm.2080. [PubMed: 20081862]

Fares I, Chagraoui J, Gareau Y, Gingras S, Ruel R, Mayotte N, Csaszar E, Knapp DJHF, Miller P, Ngom M, Imren S, Roy D-C, Watts KL, Kiem H-P, Herrington R, Iscove NN, Humphries RK, Eaves CJ, Cohen S, Marinier A, Zandstra PW, Sauvageau G, 2014. Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science (80-.) 345 (6203), 1509–1512. 10.1126/science:1256337.

Ieyasu A, Ishida R, Kimura T, Morita M, Wilkinson AC, Sudo K, Nishimura T, Ohehara J, Tajima Y, Lai C-Y, Otsu M, Nakamura Y, Ema H, Nakauchi H, Yamazaki S, 2017. An all-recombinant protein-based culture system specifically identifies hematopoietic stem cell maintenance factors. Stem Cell Rep. 8 (3), 500–508. 10.1016/j.stemcr.2017.01.015.

McIntosh BE, Brown ME, Duffin BM, Maufort JP, Vereide DT, Slukvin II, Thomson JA, 2015. Nonirradiated NOD, B6.SCID Il2rgamma−/− Kit(W41/W41) (NBSGW) mice support multilineage engraftment of human hematopoietic cells. Stem Cell Rep. 4, 171–180. 10.1016/j.stemcr.2014.12.005.

Nishimura T, Hsu I, Martinez-Krams DC, Nakauchi Y, Majeti R, Yamazaki S, Nakauchi H, Wilkinson AC, 2019. Use of polyvinyl alcohol for chimeric antigen receptor T-cell expansion. Exp. Hematol 80, 16–20. 10.1016/j.exphem.2019.11.007. [PubMed: 31874780]

Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE, 2011. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science (80-.) 333 (6039), 218–221. 10.1126/science:1201219.

Papa L, Djedaini M, Hoffman R, 2020. Ex vivo HSC expansion challenges the paradigm of unidirectional human hematopoiesis. Ann. N. Y. Acad. Sci 1466 (1), 39–50. 10.1111/nyas.v1466.110.1111/nyas.14133. [PubMed: 31199002]

Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, Duarte RF, Dufour C, Kuball J, Farge-Bancel D, Gennery A, Kroger N, Lanza F, Nagler A, Sureda A, Mohty M, 2016. Hematopoietic stem cell transplantation in Europe 2014: more than 40 000 transplants annually. Bone Marrow Transpl. 51, 786–792. 10.1038/bmt.2016.20.

Wilkinson AC, Igarashi KJ, Nakauchi H, 2020a. Haematopoietic stem cell self-renewal in vivo and ex vivo. Nat. Rev. Genet 21 (9), 541–554. 10.1038/s41576-020-0241-0. [PubMed: 32467607]

Wilkinson AC, Ishida R, Kikuchi M, Sudo K, Morita M, Crisostomo RV, Yamamoto R, Loh KM, Nakamura Y, Watanabe M, Nakauchi H, Yamazaki S, 2019. Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. Nature 571 (7763), 117–121. 10.1038/s41586-019-1244-x. [PubMed: 31142833]

Wilkinson AC, Ishida R, Nakauchi H, Yamazaki S, 2020b. Long-term ex vivo expansion of mouse hematopoietic stem cells. Nat. Protoc 15 (2), 628–648. 10.1038/s41596-019-0263-2. [PubMed: 31915389]

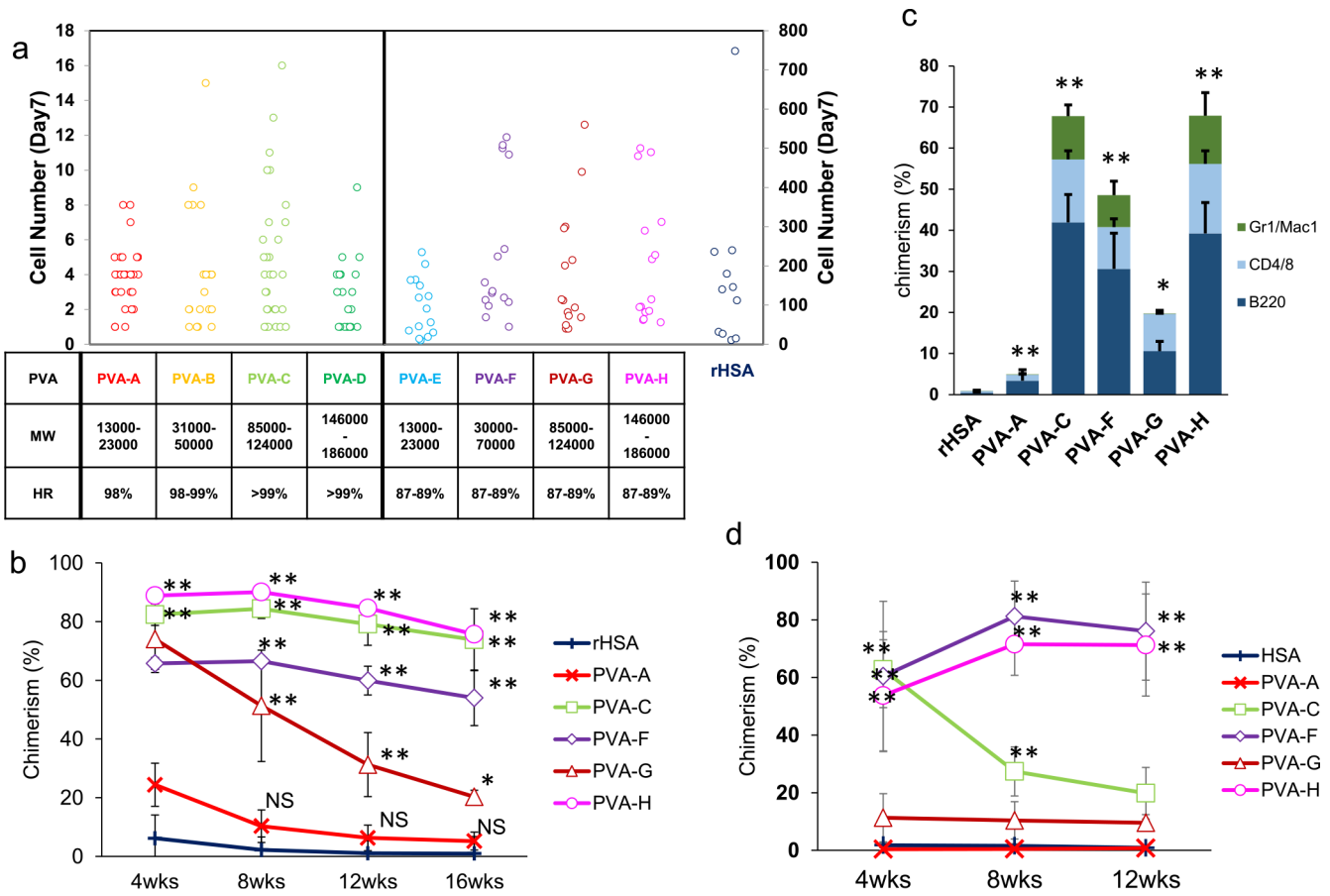

Fig. 1.

PVA chemical properties alter ex vivo expansion of functional mouse HSCs. **a**, Summary of PVA polymers used in this study and cell numbers derived from single CD34^{-}KSL mouse HSCs after seven days culture in various PVA containing media (PVA-A to PVA-H). Cell count was performed at day 7 of culture. **b**, Competitive transplantation of culture-expanded cells started from 100 HSCs against to 1×10^6 whole bone marrow (BM) cells. Donor peripheral blood (PB) chimerism in recipient mice at 4 to 16 weeks post-transplantation were analyzed by flow cytometry. Statistical significance was calculated using analysis of variance (ANOVA, Tukey-Kramer). *$p < 0.05$, **$p < 0.01$; NS, not significant. **c**, Donor PB chimerism at 16 weeks after transplantation. Multi lineage (B cell, T cell and Myeloid) reconstitution was detected in the recipient mice by using anti-CD45R (B220), anti-CD3 and anti-Gr-1/anti-Mac-1 antibodies, respectively. Statistical significance was calculated using analysis of variance (ANOVA, Tukey-Kramer). *$p < 0.05$, **$p < 0.01$; NS, not significant. d, Secondary transplantation of PVA-cultured mouse HSCs. 1×10^6 whole bone marrow cells from primary recipient mice were transferred into lethally irradiated secondary recipient mice and chimerism in recipient mice PB was analyzed over 12-weeks. Statistical significance was calculated using ANOVA and Tukey-Kramer. **$p < 0.01$.

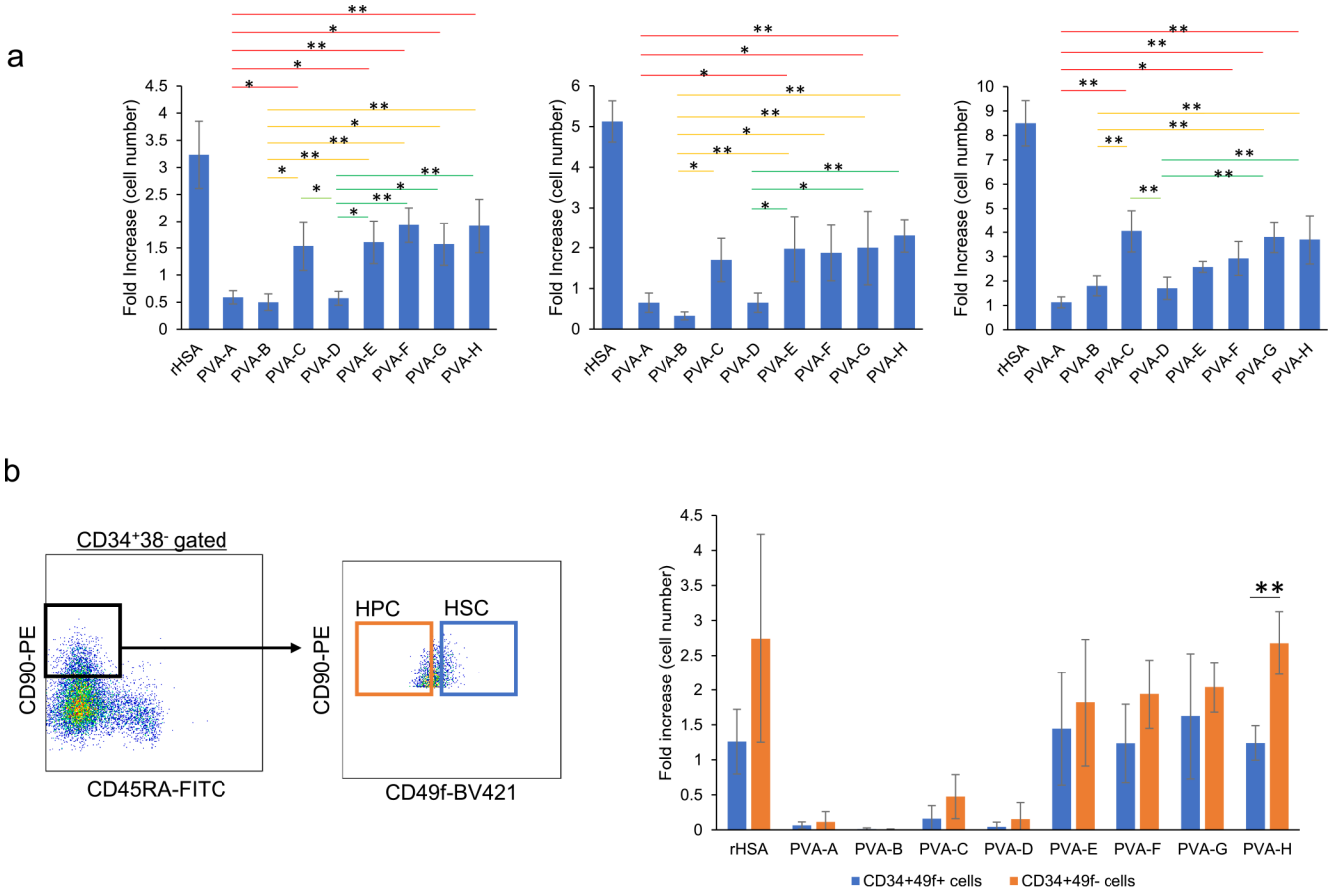

Fig. 2.

Effect of different PVA hydrolysis rates and molecular weights on ex vivo cultured human cord blood CD34$^+$ hematopoietic stem and progenitor cells. **a**, Human cord blood CD34$^+$ cells were cultured in PVA media and cell numbers were counted at day 14. Three independent experiments were performed with cord blood CD34$^+$ cells derived from different donors. Cell cultures were started from 7×10^3 (Exp.1) or 5×10^3 (Exp. 2 and 3) CD34$^+$ cells. Mean ± S.D is shown. Statistical significance was calculated using ANOVA followed by Tukey-Kramer; *$p < 0.05$, **$p < 0.01$. **b**, Proliferation of the two different CD34$^+$ cell fractions after 7-day culture in PVA-containing media. Fifty cells were sorted into four wells in a 96-well plate and cell numbers in each well were counted at 7-day. Statistical significance was calculated using ANOVA and Tukey-Kramer. **$p < 0.01$.

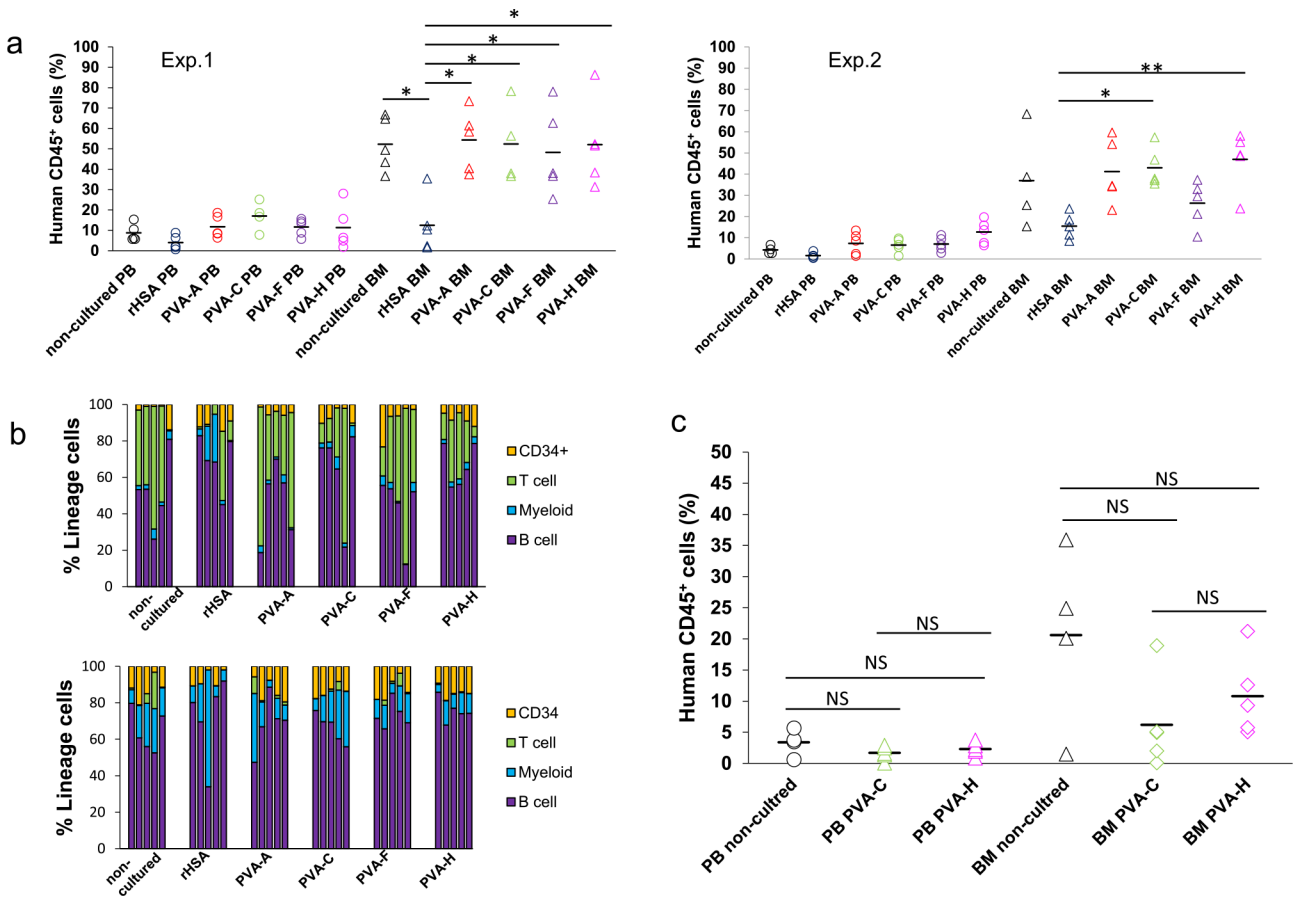

Fig. 3.

Effect of PVA HR and MW on ex vivo human $CD34^+$ engraftment potential. **a**, Mean human $CD45^+$ PB and BM chimerism at 16 weeks post-transplantation. Culture expanded human hematopoietic cells derived from 1×10^4 $CD34^+$ cells in 7-day-culture were transplanted into sub-lethally irradiated NOG (NOD.Cg-Prkdc^{scid} Il-2rγ^{null}/SZJ) mice (n = 5 mice per group). Statistical significance was calculated using ANOVA followed by Tukey-Kramer; *p < 0.05, **p < 0.01. Exp: Experiment. PB: Peripheral Blood. BM: Bone Marrow. NS: Not Significant. b, The frequency of B, T, myeloid and $CD34^+$ cells in human $CD45^+$ cells in PB (upper) and BM (lower) at 16 weeks post-transplantation. Anti-CD19, -CD3, -CD33 and -CD34 antibodies were used to detect B, T, myeloid and $CD34^+$ cells, respectively. **c**, Transplantation of 1×10^4 non-cultured $CD34^+$ cells or 7-day cultured hematopoietic cells into non-irradiated NOG mice (n = 5 mice per group). For each NOG mouse, non-cultured $CD34^+$ cells (1×10^4 cells) or the cells expanded from 1×10^4 $CD34^+$ cells were transplanted from one UCB donor (see Fig. S3 for biological replicate). Statistical significance was calculated using ANOVA followed by Tukey-Kramer; PB: Peripheral Blood. BM: Bone Marrow. *P < 0.05, NS: Not Significant.

Key Resources Table

Reagent or resource	Source	Identifier
Antibodies		
PE-Cy7 anti-mouse CD45.1	BioLegend	Cat# 110730
BrilliantViolet421 anti-mouse CD45.2	BioLegend	Cat# 109832
PE anti-mouse CD11b (M1/70)	eBioscience	Cat# 12-0112-82
APC-eFluor780 anti-mouse CD45RA (RA3-6B2)	eBioscience	Cat# 17-0452-83
APC anti-mouse CD4 (RM4-5)	BioLegend	Cat# 100516
APC anti-mouse CD8 (536.7)	eBioscience	Cat# 17-0081-83
FITC anti-human CD45RA	BioLegend	Cat# 304106
PE anti-human CD90	BioLegend	Cat# 328110
APC anti-human CD34	BioLegend	Cat# 343607
PE-Cy7 anti-human CD38	BD	Cat# 335790
Brilliant Violet421 anti-human CD49f	BD	Cat# 562582
PE-Cy7 anti-human CD45	eBiosciences	Cat# 25-0451-82
FITC anti-human CD45	BD	Cat# 555482
PE anti-human CD19	BD	Cat# 555413
APC anti-human CD33	BD	Cat# 551378
APC-Cy7 anti-human CD3	BioLegend	Cat# 344818
Brilliant Violet421 anti-human CD34	BD	Cat# 562577
Human cord blood CD34+ cells	RIKEN BRC	
Human cord blood CD34+ cells	StemExpress	Cat# CB3401C
Chemicals, Peptides, and Recombinant Proteins		
Recombinant human serum albumin	Albumin Biociences	Cat# 1002
Recombinant mouse thrombopoietin	Peprotech	Cat# 315-14
Recombinant mouse stem cell factor	Peprotech	Cat# 250-03
PVA	Sigma	Cat# 348406, 363146, 363103, P8136, 363170, 363081, 363065, 363138
Recombinant human thrombopoietin	R&D	Cat# 288-TPN/CF
Recombinant human stem cell factor	R&D	Cat# 255-SC
BellCurve for Excel	Social Survey Rearch Information Co., Ltd	
Other		