CH02 Peptide Promotes CD34+ Umbilical Cord Blood Hematopoietic Stem Cells Ex Vivo Expansion

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Abstract

Background: Umbilical cord blood (UCB) has been clinically used for human hematopoietic stem cells (HSCs) transplantation. However, limited numbers of the functional UCB-HSCs from single cord blood restricts its application in adults, while most of the strategies for stem cells expansion in vitro are either inefficient or costly. To overcome these obstacles, we evaluated the potential role of our newly identified CH02 peptide in ex vivo culture expansion of CD34+ UCB-HSCs.

Methods: Enriched human CD34+ progenitor/stem cells populations were cultured in serum-free medium supplemented with different cytokines combinations for 8 days. These cytokines combinations included various concentration of CH02 peptide or the FLT3 ligand, with a cocktail of several growth factors such as IL-6, SCF and TPO. In addition, the global gene expression profile of the CD34+ cells cultured under different conditions were monitored through RNA-seq experiments. Furthermore, the expanded CD34+ cells were topically transplanted into the dorsal wounds of diabetic mice, and the wound closure was observed to evaluate the pro-repair ability of CH02-cultured CD34+ cells.

Results: We herein report that the combination of CH02 peptide and other cytokines under the serum-free medium can effectively expand the CD34+ HSCs into 12-fold within 7 days while maintaining their stem cell properties. Moreover, CH02 peptide increased the anti-inflammatory and growth-promoting capacity of CD34+ cells, and thus accelerating wound healing of diabetic mice via promoting the anti-inflammatory and inhibiting the inflammatory factors.

Conclusions: Together, our CH02 peptide demonstrated promising potentials to improve HSCs expansion for clinical application.

Background

Hematopoietic stem cells (HSCs) transplantation is curative for many malignant blood diseases. Nowadays, sources of HSCs for transplantation are mainly from bone marrow (BM), mobilized peripheral blood (mPB), and umbilical cord blood (UCB) [1, 2]. Among them, UCB is an ideal source for HSCs transplantation due to its easy availability, low immunogenicity and low incidence of graft versus host disease (GVHD) [3, 4]. However, the amounts of HSCs from single unit umbilical cord blood, especially the absolute number of CD34+ cells, is very limit [5], which hinder the application of cord blood HSCs.

Ex vivo expansion of UCB-HSCs is a promising possibility to generate sufficient numbers of CD34+ cells for HSCs transplantation [6–8]. Plenty of studies have shown that co-culture with stromal cells [7, 9, 10], with various cytokines, such as stem cell factor (SCF) [11, 12], thrombopoietin (TPO) [13], Fms-related tyrosine kinase 3 ligand (FLT3 L) [14–16] and interleukin (IL)-3, -6, -11 [17, 18], or with small molecules and chemicals like tetraethylenpentamin (TEPA) [19] can realize the survival and self-renewal of HSCs in vitro. However, most of the strategies for stem cell expansion in vitro are either inefficient or costly, and thus, efficiency optimization and cost reduction are still needed.

Our previous study had identified a peptide with 7 amino acid residues, namely CH02, can activate FGFR signaling and promote recovery from dorsal root crush injury [20]. Through target capture, we found that CH02 can bind to receptors such as ERBB2, FLT3, MET, EGFR, PDGFR, and FGFR2, among which FLT3 and CH02 shown the highest binding affinity and abundance. Notably, FLT3 and its ligand FLT3L had been proved to stimulate the growth of the primitive hematopoietic progenitor cells in bone marrow and blood [16, 21]. Based on this, we speculated that CH02 peptide might also activate FLT3 and played a role in the in vitro expansion of hematopoietic stem cells.

In the present study, we evaluated the potential role of CH02 in ex vivo culture expansion of CD34+ HSCs. We found that combination of CH02 peptide and other cytokines under the serum-free medium could effectively promote the proliferation of CD34+ cells and maintain their stem cell properties. Moreover, CH02-cultured UCB-CD34+ cells significantly promoted the wound healing in diabetic mice via promoting the anti-inflammatory and inhibiting the inflammatory factors. Therefore, our CH02 peptide might functioned as a new and cost-efficient cytokine that could improve the expansion of UCB-HSCs for potential clinical applications.

Methods

Isolation and purification of CD34+ progenitor/stem cell populations from the umbilical cord blood

Following informed consent and with the approval of the Ethics Committee at the Ji-nan University, the umbilical cord blood was collected from normal full-term delivery in the First Affiliated Hospital of Jinan University. Mononuclear cells (MNCs) were isolated by Ficoll-Hypaque gradient centrifugation, from which CD34+ cells were selected using Miltenyi-MACS CD34+ selection kit (Miltenyi, 130-046-702) according to the manufacturer's instructions. The information of cord blood samples and frequency of CD34+ cells after enrichment were showed in Additional file 1: Table S1.

Ex vivo culture of CD34+ cells in serum-free medium

CD34+ enriched cells from cord blood were seeded at 5 × 10^4 cells/ml in SFEM medium (09650, Stem Cell Technologies) with different cytokines combinations for 8 days, and half of the medium was replaced every 48 hours. Different cytokines combinations in SFEM medium included a cocktail of several growth factors of 20 ng/ml Interleukin 6 (PeproTech, 200-06-5), 100 ng/ml of SCF (PeproTech, 300-07-5), and 100 ng/ml TPO (PeproTech, 300-18-50) with or without 100 ng/ml FLT3 ligand (PeproTech, 300-19-10) and the indicated concentration of CH02 peptide as follow:

SIT: SFEM supplemented with SCF, TPO and IL6.

SITF: SFEM supplemented with SCF, TPO, IL6 and FLT3L.

SITH (5): SFEM supplemented with SCF, TPO, IL6 and 5ng/ml CH02.

SITH (10) or SITH: SFEM supplemented with SCF, TPO, IL6 and 10ng/ml CH02.

SITH (15): SFEM supplemented with SCF, TPO, IL6 and 15ng/ml CH02.

SITH (20): SFEM supplemented with SCF, TPO, IL6 and 20ng/ml CH02.

For all animal experiments described below, the expanded CD34+ cells were used directly after 7–8 days culture in the indicted SFEM medium.

Flow cytometry analysis

Enriched UCB-CD34+ cells or CD34+ cells cultured in different cytokines combinations SFEM medium for 7 days were harvested, and the total nucleated cells counted via trypan blue staining. To analysis the cell composition of CD34+ cells, flow cytometry staining was performed with CD34- FITC and CD38-APC antibodies (BD Biosciences, Burlington, Canada), then samples were subjected to flow cytometer analysis using a FACScan flow cytometer (Beckman Coulter, USA) (Additional file 2: Fig. S1). For each sample, at least 50,000 events were recorded, and the isotype controls were used in all cases. All samples were labeled for 30 minutes at 4°C, washed, and fixed in 10% formalin according to the manufacturer's instructions

Colony-forming unit (CFU) assay of CD34+ cells

Enriched UCB-CD34+ cells and CD34+ cells cultured in different cytokines combinations medium for 7 days were seeded at 1000 cells/ml in semisolid culture (Stem Cell Technologies, H4434) following the manufacturer’s instructions for the colony-forming unit assay. After incubation at 37 °C under 5% CO2 at 100% humidity for 14 days, the burst-forming unit-erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), colony-forming unit-granulocyte/macrophage (CFU-GM), and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM) levels were scored under an inverted microscope. The numbers of CFU per 1000 UCB-CD34+ cells before cultivation (day 0) and after harvest (day 7) were calculated.

Dorsal mouse wound models for transplantation

All animal experiments were conducted following protocols approved by the Institute of Laboratory 163 Animal Science, Jinan University, China. The db/db male mice (BKS-Leprem2Cd479/GptStock No: T002407, Jackson Labs, Wilmington, USA) were purchased from Nanjing Biomedical Research Institute at 6 -7 weeks of age and left for 2 weeks to acclimatize, and the cages were changed daily due to excessive urination caused by diabetes symptoms. The mouse was induced and maintained on 3% pentobarbital sodium anesthesia (5mg/kg), and the dorsal surface was shaved, and the fur were completely removed by a depilatory cream. The donut shaped splint was fix to the surrounding skin by bonding adhesive and 4 6-0 silk, and the full thickness wound was created by a sterile disposable 6 mm biopsy punch. About 2 × 10^5 cells were injected to the surface of the wounds (overlay) or around the wound edge then covered with 20 μl of fibrin, while the control wounds were injected with PBS.

Measurement of the wound area

Wound area was monitored with a scale every 48h by taking photos using digital camera for 21 days. The silicon splints were used as a size reference when analyzing. Pictures were analyzed by drawing around the wound margins and measuring pixel area using Image J.

RNA sequencing and data analysis

RNA samples were prepared from UCB-CD34+ cell cultured under different conditional medium. Total RNA was extracted using an RNAisoPlus kit (TAKARA). After extraction, mRNA was enriched and fragmented, and then reversed transcription into cDNA with random primers. Subsequently, the cDNA fragments were purified with a QiaQuick PCR extraction kit (Qiagen, Netherlands), end-repaired and ligated to sequencing adapters. The cDNA libraries were sequenced on the Illumina sequencing platform by Novogene Biotechnology Co., Ltd. raw reads were filtered by fastp v0.18.0 to get high quality clean reads [22]. Paired-end clean reads were used for mapping to genome with HISAT 2.2.4 [23]. Then the mapped reads of each individual sample were assembled using StringTie v1.3.1 in a reference-based approach [24]. For each transcription region, fragment per kilobase of transcript per million mapped reads (FPKM) value was calculated to quantify its expression abundance and variations. Differential genes expression analysis was conducted by DESeq2 software [25]. Genes with a parameter of false discovery rate (FDR)$\leq$0.05 and absolute fold change$\geq$2 were filtered as DEGs. Then, DEGs were mapped to GO terms in the GO database (http://www.geneontology.org/), and gene numbers were calculated for each time. Significantly enriched GO terms in DEGs compared with the background were defined by a hypergeometric test. Target gene set pathway analysis conducted with Ingenuity Pathway Analysis software (Qiagen) [26].

Statistical analysis

The statistical differences between each group were analyzed using the GraphPad 9.0 Prism statistical software for all the experiment data. The comparison was analyzed be-tween two groups with the student t test. The values were plotted as mean ± standard deviation. Probability values *$p \leq 0.05$ was considered as statistically significant difference, and ***$p \leq 0.01$ was considered as highly significant difference.

Results

CH02 peptide promoted CD34+ Umbilical cord blood Hematopoietic Stem cells (UCB-HSCs) ex vivo expansion

The CD34+ cells were isolated through gradient centrifugation followed by immunomagnetic beads sorting from UCB, and our data shown that CD34+ cells from 10 UCB units had an average frequency of 65% (±13%) (Table S1 and Additional file 2: Fig. S1). This included both the CD34+/CD38− and CD34+/CD38+ cells, and the CD34+/CD38+ represented the committed progenitors has a percentage of 82%, while the CD34+CD38- represented the multipotent progenitors were only 1.5% (Additional file 2: Fig. S1 and Additional file 5: Fig. S4C). Previous studies showed that CD34+ cells can be cultured in StemSpan medium (Stem Cell Technologies) supplemented with FLT3L, SCF, IL-6, and TPO [27]. Among

them, FLT3L is the ligand of FLT3, which is expressed in the human CD34+ progenitor cell compartment to promote the survival of HSCs [16, 21]. Our previous study had identified CH02 peptide could activate FGFR signaling and promote the recovery of dorsal root crush injury (Additional file 3: Fig. S2) [20]. Through target capture, we found that CH02 could bind to receptors such as ERBB2, FLT3, MET, EGFR, PDGFR, and FGFR2 (Additional file 4: Fig. S3A), among which FLT3 and CH02 shown the highest binding affinity and abundance (Additional file 4: Fig. S3B). The multiple targeting of CH02, especially its targeting to FLT3 and function in injury repair, suggesting that this peptide might have a potential function in the CD34+ cells expansion.

To explore the function of CH02 on the proliferation of CD34+ UCB-HSCs, we performed 7-day cultures under different conditions described above (SIT, SITF, SITH (5), SITH (10), SITH (15), SITH (20)) (Fig. 1). As shown in figure 1A, the total numbers of the UCB-HSCs were largely increased under all culture conditions compared with the fresh group without culture. Among them, the SITF group showed the highest proliferation rate, which was the 18-fold of the fresh group, while the SIT, SITF, SITH (5), SITH (10), SITH (15) and SITH (20) group were about 11-12 folds higher than that of the fresh group (Fig. 1A). The frequency of CD34+ cells was declining on all culture conditions, while the frequency of CD34+ cells in the SITH (10) group and SITH (15) group were nearly 8 percent higher that of the SITF group, suggesting that CH02 peptide was more effectively than FLT3L to inhibit the CD34+ cells differentiation while promoting proliferation (Fig. 1B and Additional file 5: Fig. S4A). In addition, the frequency of CD38+ cells were declining on all culture conditions (Additional file 5: Fig. S4B and S4D), leading to a lower frequency of CD34+/CD38+ committed progenitors (Fig. 1C, Additional file 5: Fig. S4C and S4E) and a higher ratio of CD34+/CD38- multipotent progenitors (Fig. 1D, Additional file 5: Fig. S4C and S4E), suggesting that ex vivo culture under these conditions could effectively promote the self-renewal and proliferation of pluripotent stem/progenitor cells (Fig. 1C and 1D). Notably, the frequency of both CD34+/CD38+ and CD34+/CD38- in the SITH (10) groups were a little higher that of the SIT and SIFT group (Fig. 1C and 1D). These results indicated that although FLT3L could promote the efficient proliferation of CD34+ cells, our CH02 peptide was able to maintain more stem cell properties while promoting proliferation.

CH02 peptide promoted the proliferation of committed progenitors

Hematopoietic stem cells (HSCs) are capable of self-renewal and differentiation into all blood cell types. To explore the function of CH02 on the proliferation of committed progenitors, we calculated the cloning numbers of the committed progenitors formed by the initial 1000 CD34+ cells under different culture conditions via colony-forming assays (CFC assays) for 14 days. Our results showed that the cloning numbers of the CFU-GM and CFU-E/BFU-E under SITF and SITH culture conditions were significantly higher than their counterparts ($p < 0.01$), while there was no significant difference ($p > 0.05$) between these two groups, although the total number of clones in SITH was higher than that in the SITF group (Fig. 2). These results suggested that our CH02 peptide could promote the proliferation of committed progenitors.

CH02 peptide increased the anti-inflammatory and growth-promoting capacity of UCB-CD34+ cells

Different culture conditions might affect the gene expression patterns and thus regulating their cellular functions. The results presented so far demonstrate that our CH02 peptide can maintain multipotency of the CD34+ cells while promoting proliferation. To monitor the global gene expression change during proliferation, we performed RNA-seq experiments of the CD34+ cells on different culture conditions. Pearson correlation coefficient of gene expression profiles showed the association among different samples, and our results showed that the correlation coefficients between each two samples of the SIT group, SITF group and SITH group were all higher than 0.995 (Fig. 3A), indicating that the expression profiles of the three samples were highly similar.

Despite this, we still found several differentially expressed genes (DEGs) among these 3 groups (Fig. 3D and E). For example, the expression of the FLT3 gene was the highest in the SITF group, while its expression in the SITH group was higher than that in the SIT group, indicating that the addition of CH02 peptide can stimulate the expression of FLT3 (Fig. 3B). Moreover, we found that the expression of CD34 gene was the highest in the SITH group, which was consistent with the previous flow analysis results (Fig. 3C); while other hematopoietic stem cells markers, such as CD49f and ESAM (Additional file 6: Fig. S5C and S5D), was significantly higher in the SITH group than in the SITF group, suggesting that CH02 peptide maintained the higher stem cell properties of CD34+ cells comparing to FLT3L.

In addition, we found 72 differentially expressed genes (DEGs) between the SITH group and the SITF group (Fig. 3D and 3E). We then used the IPA (Ingenuity Pathway Analysis) to perform the KEGG enrichment analysis, and our result showed that the up-regulated genes in SITH group were mainly enriched in anti-inflammatory and cell growth signaling pathways such as TGF-β signaling, PAK signaling, PDGF signaling and GM-CSF signaling (Fig. 3F), which were related to wound healing; while the down-regulated genes were mainly enriched in IL-6 signaling, IL-8 signaling, NF-κB signaling and other pathways related to inflammation (Fig. 3F), suggesting that UCB-CD34+ cells cultured in SITH condition might reduce inflammation and promote cell growth.

CH02 peptide increased the capacity of UCB-CD34+ cells in promoting wound Healing

Previous studies showed that the expanded UCB-CD34+ cells could accelerate the wound healing of the diabetic mice [28], while our RNA-Seq result showed that CH02 peptide might increase the anti-inflammatory and growth-promoting capacity of UCB-CD34+ cells compared to the FLT3L. Similarly, we used the db/db mice as diabetic skin ulcers model to evaluate the pro-repair capacity of CD34+ cells after expansion under STIH culture condition (Fig. 4A). Our results showed that both wild-type and diabetic mice showed inflammation after the establishment of dorsal trauma in mice (Additional file 7: Fig. S6). The inflammation of wild-type mice began to subside on day 4, while that of diabetic mice lasted until day 14, indicating that wound healing in diabetic mice was slow (Additional file 7: Fig. S6). Compared with the PBS group, the injection of SITF-cultured or SITH-cultured UCB-CD34+ cells largely accelerated wound healing in diabetic mice (Fig. 4B-D). Notably, SITH-cultured UCB-CD34+ cells had better healing effect than that of the SITF-cultured group (Fig. 4B-D), indicating that CH02 peptide might increase, at least maintain, the capacity of UCB-CD34+ cells to promote wound healing. In line with this, inflammatory

factors such as iNOS were significantly reduced on day 10 after injection of the SITH-cultured UCB-CD34+ cells, while the expression levels of anti-inflammatory factors in the same condition were increased (Fig. 4E), especially the expression level of TGFβ1, which was increased by 15-fold compared to the PBS group, suggesting that the wound inflammation of diabetic mice began to subside after topical transplantation

Discussion

Multiple approaches have been employed for ex vivo expansion of UCB-HSCs by using combinations of specific media, cytokines and even the hematopoietic stromal cells as feeder layer. Ueda et al reported that combinations of cytokine SCF, FLT3L, TPO, IL-3, IL-6 and s-IL-6R in 20% FBS containing medium can successfully expand UCB-CD34+ cell into 4.2-fold [29], while Li et al found that combinations of SCF, FLT3L and TPO can efficiently expand and maintain the UCB-CD34+ cells under the condition of WJ-MSCs or UVECs feeder layer serum-free co-cultivation [7]. In this study, our CH02 peptide was able to combine with other cytokines under the serum-free medium to effectively expand the CD34+ HSCs into 12-fold while maintaining their stem cell properties up to 7 days.

FLT3 is a receptor tyrosine kinase (RTK) that mainly expressed in the primitive hematopoietic progenitor cells [21]. Through interaction with FLT3L, FLT3 activates the downstream PI3K and RAS pathways to stimulate the growth of progenitor cells in bone marrow and blood[16, 21]. In this study, we found that CH02 can also target to FLT3 (Additional file 3: Fig. S2B and S2B), and thus, we suspected that CH02 peptide might also activate the RTK pathway to promote the expansion of HSCs in vitro, which is similar to the function of FLT3L. In consistent with this, we found that both FLT3L and CH02 could stimulate the expression of FLT3 (Fig. 3B), and the expression profile of CD34+ cells under STIF and STIH condition were highly similar (Fig. 3A). Nevertheless, we still found slight differences between the two Ligands. FLT3 seemed to be more conducive to the expansion of CD34+ cells (Fig. 1A), while our CH02 peptide could maintain more pluripotency while promoting proliferation (Fig. 1B and 1D). Therefore, CH02 peptide might function as a new and cost-efficient cytokine to replace the commercial FLT3L for ex vivo expansion of CD34+ UCB-HSCs.

People with type 2 diabetes are suffering from chronic and nonhealing wounds. Using db/db mice as diabetic skin ulcers model, Whiteley et al reported that culture-expanded CD34+ cells from frozen umbilical cord blood could accelerate the wound healing and full thickness skin regeneration [28]. Similarly, our results showed that the both SITF-cultured and SITH-cultured CD34+ cells could also accelerate the wound healing and improved skin regeneration in db/db mice (Fig. 4B-D). Notably, CD34+ cells cultured in SITH condition have a better healing effect than that of the SITF-cultured condition (Fig. 4B-D), which also proved that our CH02 peptide was superior to FLT3L in the ex vivo expansion of CD34+ cells. Previous studies showed that UCB-CD34+ cells are potential sources of therapeutic endothelial precursor cells (EPC) and circulating angiogenic cells (CAC) [30], which displayed powerful paracrine signals that promote tissue regeneration. Due to the incompatibility between db/db mouse diabetic wounds and human donor cells, we speculated that our expanded UCB-CD34+ cells may promote

wound healing in db/db mice by secreting a variety of chemokines and cytokines. In line with this, our RNA-Seq result showed that SITH-cultured CD34+ cells were more effectively to reduce inflammation and promote cell growth compared with the SITF-cultured cells (Fig. 3F). Furthermore, we had detected that inflammatory factors such as iNOS in wounds were significantly reduced after implantation of the expanded UCB-CD34+ cells, while the expression of anti-inflammatory factors such as TNF-α, IL-1β and TGFβ1 were increased gradually (Fig. 4E). Although more evidence is needed, these observations are consistent with Whiteley's report that UCB-CD34+ can promote tissue repair by secreting anti-inflammatory, pro-vascular and anti-apoptotic factors [6]. Collectively, these findings demonstrated promising potential for the CH02 peptide to improve UCB-HSCs expansion for clinical application.

Conclusions

In conclusion, our results demonstrated that CH02 peptide greatly enhanced the expansion of CD34+ HSCs while maintaining their stem cell properties. Moreover, topical transplantations of the SITH-cultured CD34+ cells accelerated the wound healing of diabetic mice strongly suggested that CH02 peptide might be efficacious in potential clinical applications.

Abbreviations

UCB: Umbilical cord blood; HSCs: Hematopoietic stem cells; GVHD: Graft-versus-host disease; FBS: Fetal bovine serum; SCF: Stem cell factor; TPO: Thrombopoietin; FLT3: Fms-related tyrosine kinase 3; FLT3 L: Fms-related tyrosine kinase 3 ligand; IL-6: Interleukin-6; TEPA: Tetraethylenpentamin; BFU-E: Burst-forming unit-erythroid; CFU: Colony-forming unit; CFU-E: Colony-forming unit-erythroid;CFU-GM: Colony-forming unit-granulocyte/ macrophage; CFU-GEMM: Colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte; FPKM: Fragment Per Kilobase of transcript per Million mapped reads; EPC: Therapeutic endothelial precursor cells; CAC: Circulating angiogenic cells; iNOS: Inducible nitric oxide synthase; TNF-α: Tumor necrosis factor alpha; TGFβ1: Transforming growth factor-β1; DEGs: Differentially expressed genes.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Animal Experiments of Jinan University, Guangzhou, PR China, Jinan University approved all experiments conducted in this study (ethical permit IACUC-20180620-03).

Consent for publication

Not applicable.

Availability of data and materials

Data supporting the findings of this work are available within the paper and its supplementary information files.

Competing interests

Xiaojia Chen and An Hong are the inventors of a pending patent on CH02 peptide. The remaining authors declare no potential conflict of interests.

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Authors' contributions

YY and BZ analyzed the data and wrote the manuscript. BZ, YC and ZS performed the experiments. JX, JZ, JL, FL and ZZ participated in data acquisition. YZ, JC and QM provided the technical support. JL and BL contributed materials and analysis tools. AH and XC led the study and contributed to conception and design, project supervision and final approval of the manuscript. All authors read and approved the final manuscript.

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Figures

Fig. 1

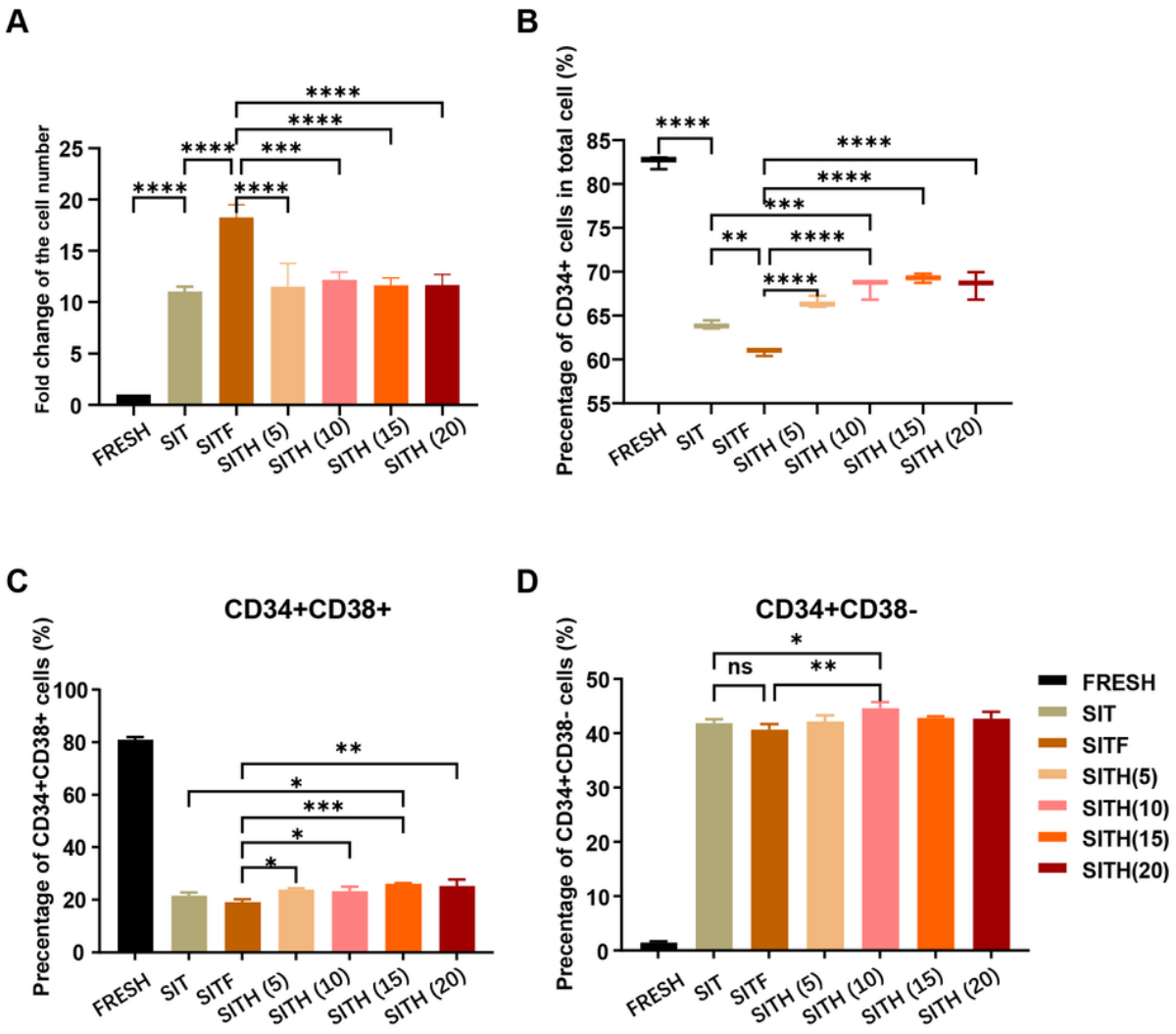

Figure 1

Expansion of UCB-CD34+ cells under different culture conditions.

(A) Total ex-pansion folds of UCB-CD34+ cells under indicated culture conditions. (B-D) Flow cy-tometric analysis of the frequency of CD34+ cells (B), CD34+CD38+ cells (C) and CD34+CD38- cells (D) under indicated culture conditions on day 0 and 7. FRESH repre-sented CD34+ cells isolated from UCB on day 0, while the SIT, SITF and SITH represented CD34+ cells under indicated culture conditions on day 7 as

described above. Data are shown as mean ± SD, n = 3. *p < 0.05, **p < 0.01 and ***p < 0.001; one-way ANOVA fol-lowed by Dunnett's multiple comparison test.

Fig. 2

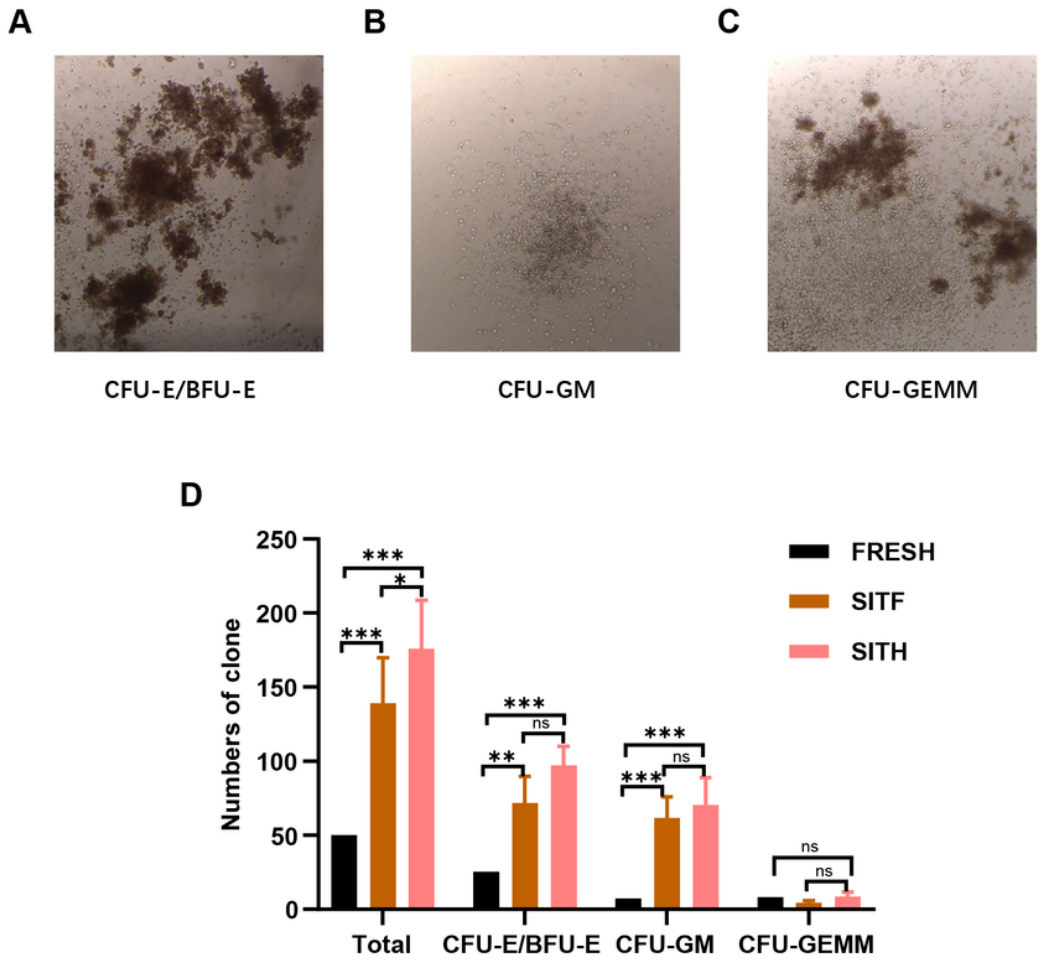

Figure 2

Expansion of committed progenitors under different culture conditions.

Morphology of CFU-E/BFU-E (A), CFU-GM (B) and CFU-GEMM (C) in different coculture conditions, and their expansion numbers (D) were measured under a microscope. CFU-E/BFU-E, colony-forming unit-erythroid/burst-forming unit-erythroid; CFU-GM, col-ony-forming unit-granulocyte/macrophage; CFU-

GEMM (C), colony-forming unit-granulocyte/erythroid/ macrophage/megakaryocyte. FRESH represented CD34+ cells isolated from UCB on day 0, while the SIT, SITF and SITH represented CD34+ cells under indicated culture conditions on day 7 as described above. Data are shown as mean ± SD, n = 3. *p < 0.05, **p < 0.01 and ***p < 0.001; one-way ANOVA followed by Dunnett's multiple comparison test.

Fig. 3

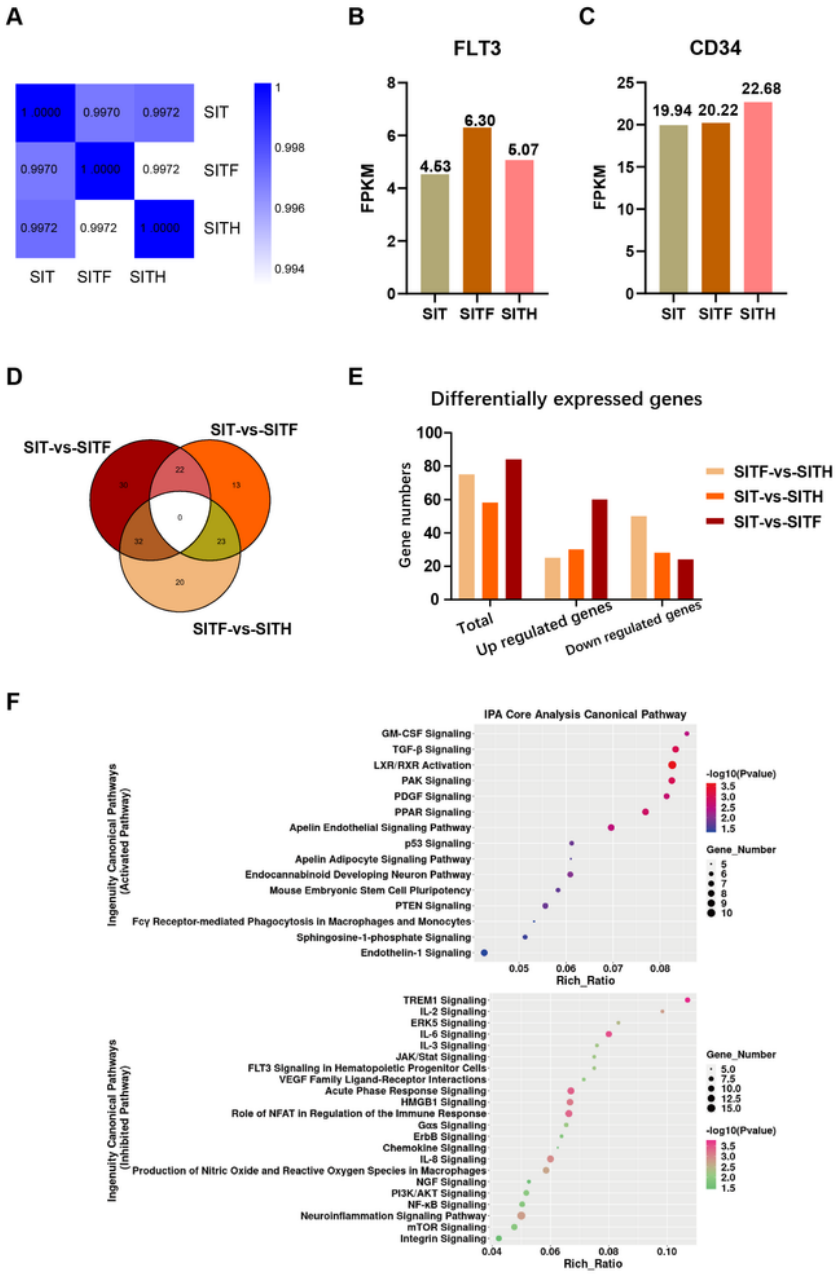

Figure 3

RNA-Seq analysis of UCB-CD34+ cells under different culture conditions.

(A) Pearson correlation coefficient of gene expression profiles of CD34+ cells under different culture conditions. SIT, SITF and SITH represented CD34+ cells under indicated culture conditions on day 7 as described above. (B and C) RNA-Seq analysis of the expression of the FLT3 (B) and CD34 (C) genes of CD34+ cells under different culture conditions. The abscissa represented the indicated culture conditions, while the ordinate represented FPKM. (D and E) Venn diagram (D)and histogram (E) showed the numbers of differentially expressed genes between each two culture conditions. (F) Bubble chart of the differentially expressed genes between the SITF and the SITH group through IPA-KEGG signal pathway analysis. The upper and lower chart show the predicted activation pathway and inhibitory pathway, respectively.

Fig. 4

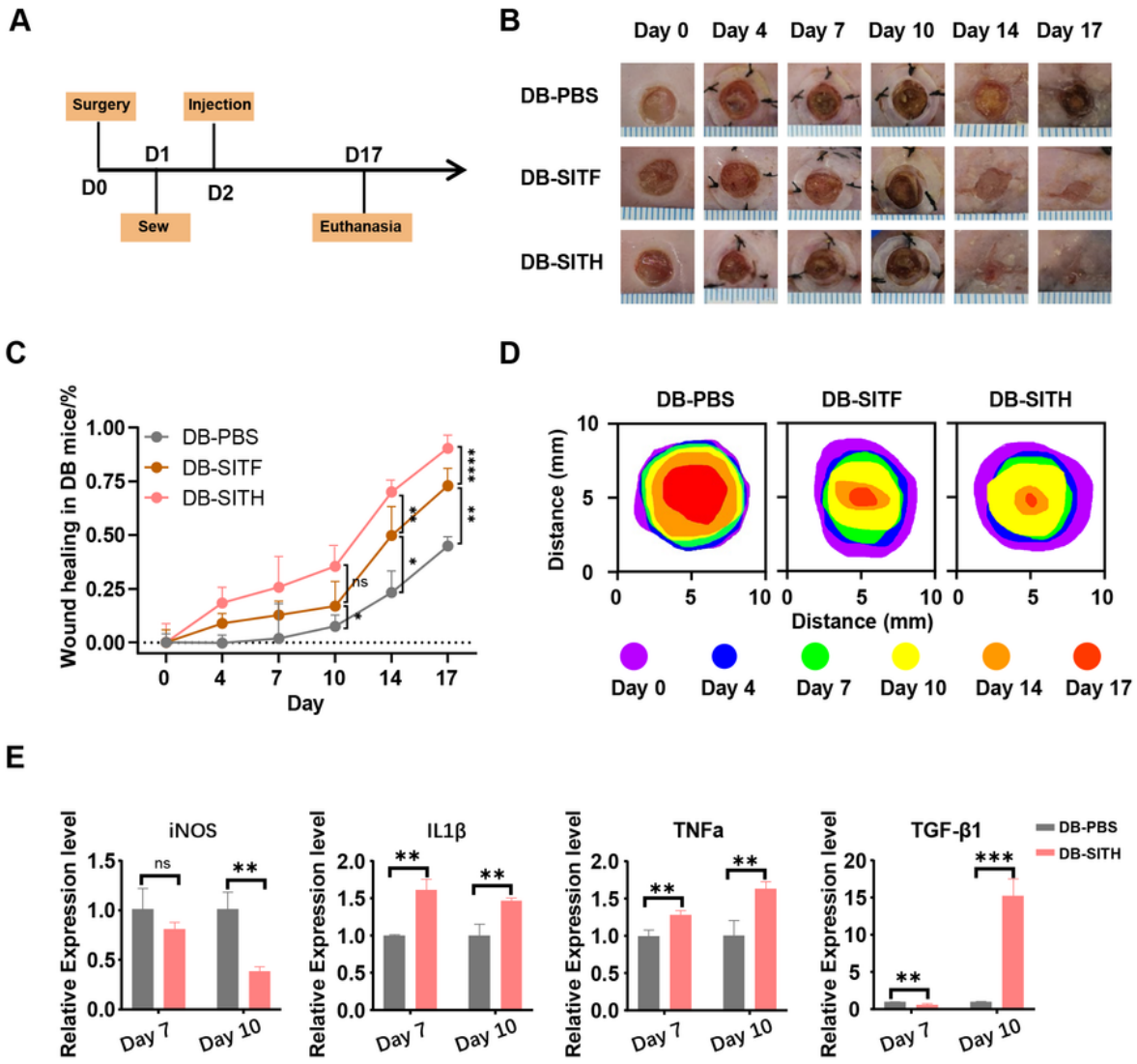

Figure 4

SITH-cultured CD34+ cells accelerated diabetic mice wounds healing.

(A) Scheme for expanded CD34+ cells transplantation in mice skin ulcers model. (B) Injured mice received the PBS or expanded CD34+ cells applied topically to the wounds. SITF or SITH represented CD34+ cells under indicated culture conditions on day 7 as described above. DB, diabetic mice. (C) Topical application

of expanded CD34+ cells in SITH group resulted (n = 5) in faster wound closure when compared to controls (n = 5) and SITF group (n = 5) (*p < 0.05). (D) Traces of wound-bed closure of the injured mice receiving the PBS or expanded CD34+ cells transplantation. (E) Expression levels of inflammatory factors and anti-inflammatory factors in wounds on day 7 and day 10. Data are shown as mean ± SD, n = 3. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.001; one-way ANOVA followed by Dunnett's multiple comparison test.

Supplementary Files

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