Differential Effects of Three Preparations of Human Serum on Expansion of Various Types of Human Cells

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Background: To avoid potential risks of animal-derived products such as viral transmission and immunologic reactions, usefulness of human-derived products in manipulation of cells for cell-based therapies has been investigated but has not yet been completely clarified.
Methods: Three types of human sera—serum from whole blood, serum from platelet-rich plasma, and serum from platelet-poor plasma—were prepared from blood samples obtained from the same four volunteers. The authors investigated the biochemical profiles of the three serum preparations as well as their potential as culture additives using three types of human cells: dermal fibroblasts, adipose-derived stem/stromal cells, and umbilical vein endothelial cells.
Results: Platelet counts differed among serum from whole blood (100 percent), platelet-rich plasma (75.1 percent), and platelet-poor plasma (12.6 percent), resulting in differential concentrations of platelet-derived growth factor and epidermal growth factor, although other biochemical values such as total protein and albumin were similar. Serum from whole blood and platelet-rich plasma highly enhanced proliferation of dermal fibroblasts compared with the effects of serum from platelet-poor plasma, but no differences in proliferative efficacy were observed in cultures of adipose-derived stem/stromal cells and vascular endothelial cells.
Conclusions: Serum from platelet-rich plasma, which is less invasive to prepare than serum from whole blood, was superior to serum from platelet-poor plasma as a substitute for animal-derived serum in culture expansion of dermal fibroblasts. Although autologous or human-derived serum preparations may be of great use in cell-based therapies, this usefulness strongly depends on the target cell species and the purpose of the cell culture. (*Plast. Reconstr. Surg.* 122: 438, 2008.)

In cell-based regenerative therapies, transplantation of cells into target tissues usually takes place after cell manipulation, but most such manipulations (e.g., cell culture) require animal-derived products such as serum or tissue extracts. Considering the risks, which include infection with viral or prion-related disease or immunologic reactions,[1] use of animal-derived products such as fetal bovine serum or bovine pituitary extract should be avoided. Thus, human-derived substances are considered the optimum materials for these manipulations. Autologous serum obtained from whole blood enhances the expansion of human mesenchymal stem cells in culture.[2–6] In contrast, preparation for clinical use of component-level plasma products, such as platelet-rich plasma or platelet-poor plasma, is considered to be less invasive because erythrocytes can be collected separately in the form of "high-density erythrocytes," which can be given back to the patients.[7,8]

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The proliferative effects in culture of various platelet derivatives such as platelet-rich plasma, platelet-released supernatant, and platelet lysates on several human cell types have been reported.[6,9–14] Our aim was to investigate the differences among three types of autologous serum—serum from whole blood, serum from platelet-rich plasma, and serum from platelet-poor plasma—in their effects on the growth of three representative replicating human cells: human adipose-derived stem/stromal cells, human dermal fibroblasts, and human umbilical vein endothelial cells. It is well known that the bioactive protein levels secreted by platelets differ substantially among donors, platelet product types, processing techniques, and methods of platelet activation.[15–21] Therefore, in this study, serum from whole blood, serum from platelet-poor plasma, and serum from platelet-rich plasma were prepared from the same four volunteers and evaluated for biochemical components and clinical potential as culture additives.

MATERIALS AND METHODS

Collection and Preparation of Plasma and Serum

Venous blood (300 ml each) was collected from four healthy volunteers—three women (26, 27, and 38 years old) and one man (44 years old) (Table 1)—after each volunteer signed informed consent approved by our institutional review board. For preparation of platelet-rich plasma and platelet-poor plasma, the methods applied clinically for the preparations of autologous blood transfusion in our facility were used. The preparation protocol and blood components of whole blood, platelet-rich plasma, and platelet-poor plasma are summarized in Figures 1 and 2. Serum was prepared from whole blood, platelet-rich plasma, or platelet-poor plasma by elimination of coagulation factors such as fibrinogen, as described below.

First, 100 ml of each blood sample was drawn into a flask, and the remaining 200 ml of blood was drawn and stored in a blood bag (CPDA; Terumo, Tokyo, Japan) containing 0.327% citric acid, 2.63% sodium citrate, 0.0275% adenine, 0.251% sodium dihydrogen phosphate, and 2.9% D-glucose solution.

Table 1. Summary of Donors

Patient	Age (yr)	Sex	Symbols in Figures
1	26	Female	○
2	27	Female	Δ
3	38	Female	□
4	44	Male	×

The blood in the flask was oscillated (agitated) at 37°C for 1 hour and incubated overnight at 4°C. The supernatant was collected using a 50-ml tube and centrifuged at 841 *g* for 10 minutes using a desktop centrifuge (KUBOTA 5200; Kubota, Co., Tokyo, Japan), and the supernatant was again collected as serum from whole blood. The stored 200 ml of blood in the blood bag was separated into 100-ml aliquots; one was centrifuged at 93 *g* for 10 minutes and the other at 841 *g* for 10 minutes. The resulting supernatants were platelet-rich plasma and platelet-poor plasma, respectively. The two types of plasma, platelet-rich plasma and platelet-poor plasma, were drawn into two flasks. After addition of 200 U of thrombin, the contents were oscillated (agitated) for 60 minutes at 37°C and incubated overnight at 4°C. The liquid component was drawn into a 50-ml tube and centrifuged at 841 *g* for 10 minutes, and the supernatants were obtained as serum from platelet-rich plasma and serum from platelet-poor plasma, respectively. The serum samples were frozen at −80°C and thawed at 37°C before analysis.

Biochemical Analysis

A small portion of whole blood, platelet-rich plasma, and platelet-poor plasma was used for biochemical analysis to investigate the number of red blood cells, white blood cells, platelets, total protein, albumin, sodium, potassium, chloride, and calcium. Analysis was performed by SRL, Inc. (Tachikawa, Japan), a commercial analysis service.

Quantitative Analysis of Platelet-Originated Growth Factors Contained in Serum

To analyze the concentration of platelet-originated growth factors in each serum sample, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) were measured using respective anti-human enzyme-linked immunosorbent assay kits (Quantikine; R&D Systems, Inc., Minneapolis, Minn.) according to the manufacturer's instructions. Levels of immunoreactive cytokines were measured at 450 nm by a microplate reader (Model 550; Bio-Rad Laboratories, Hercules, Calif.), and a standard curve was generated to determine growth factor concentrations (in picograms per milliliter).

Preparation of Human Dermal Fibroblasts

Human dermal fibroblasts were isolated from normal skin samples obtained from plastic surgery

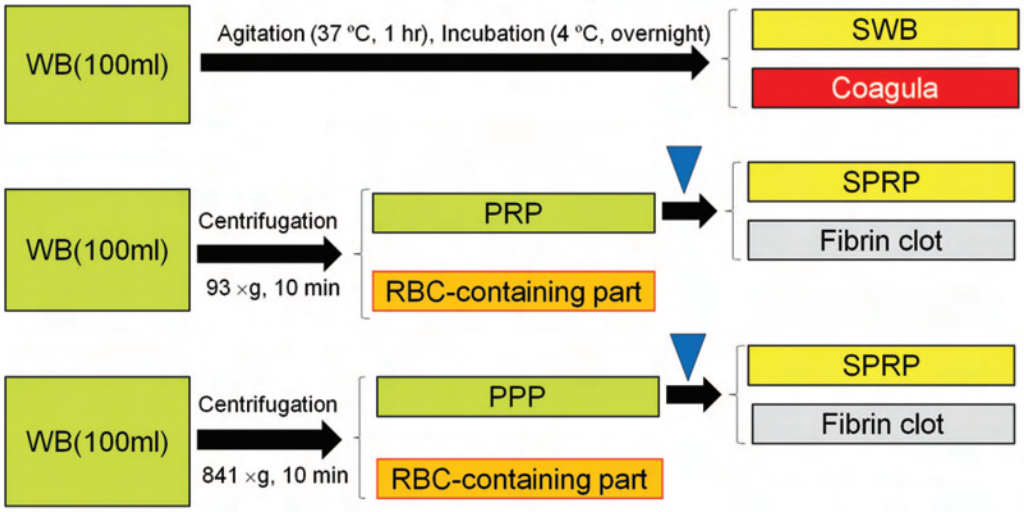

Fig. 1. Preparation of three types of human serum. For preparation of platelet-rich plasma or platelet-poor plasma, whole blood was separated by centrifugation into two components: platelet-rich plasma and the remainder (the red blood cell–containing part), or platelet-poor plasma and the remainder (the red blood cell–containing part). Serum from platelet-rich plasma and serum from platelet-poor plasma were prepared through thrombin activation (*blue triangles*) of platelet-rich plasma and platelet-poor plasma, respectively. *WB*, whole blood; *PRP*, platelet-rich plasma; *PPP*, platelet-poor plasma; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma; *RBC*, red blood cells.

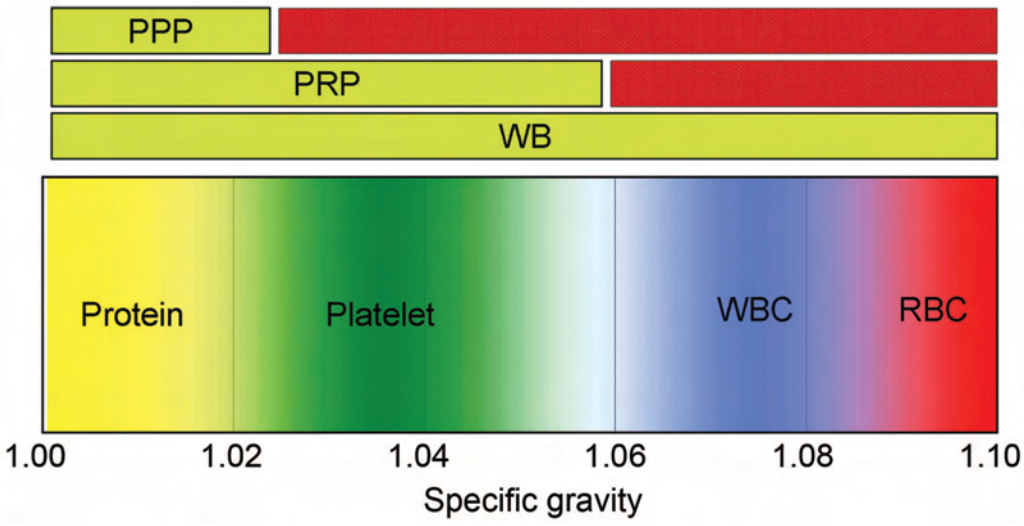

Fig. 2. Preparation of three types of human serum. Blood components differ in specific gravity. Because of the differential specific gravity, platelet-rich plasma and platelet-poor plasma can be separated from whole blood by specific centrifugation protocols. *WB*, whole blood; *PRP*, platelet-rich plasma; *PPP*, platelet-poor plasma; *WBC*, white blood cells; *RBC*, red blood cells.

after informed consent approved by the institutional review board. The skin samples were cut into pieces of approximately 3 × 3 mm and treated with 0.25% trypsin in phosphate-buffered saline solution for 24 hours at 4°C. After removal of the epidermis, the interstitial tissue fragments were attached to 100-mm plastic dishes and cultured with Dulbecco's Modified Eagle Medium (Nissui Pharmaceutical, Tokyo, Japan) culture medium containing 10% fetal bovine serum. Primary hu-

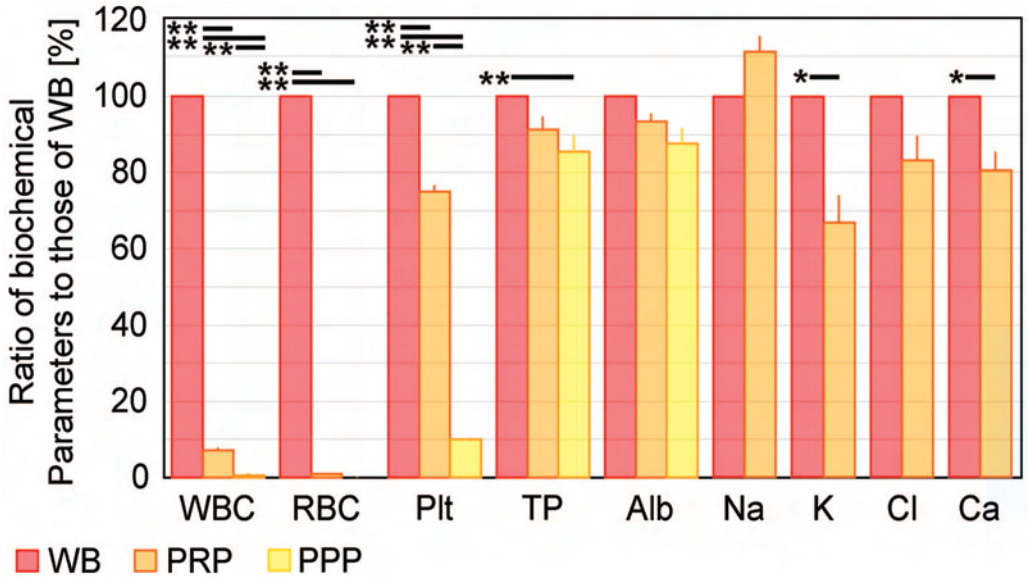

Fig. 3. Blood counts and biochemical data in whole blood, platelet-rich plasma, and platelet-poor plasma. Values are expressed as ratios of biochemical parameters to those of whole blood. Paired *t* tests were used for statistical analysis. *Black bars* above indicate statistical significance (*$p < 0.05$; **$p < 0.01$). Values are mean ± SE. *WB*, whole blood; *PRP*, platelet-rich plasma; *PPP*, platelet-poor plasma; *WBC*, white blood cells; *RBC*, red blood cells; *TP*, total protein; *Alb*, albumin.

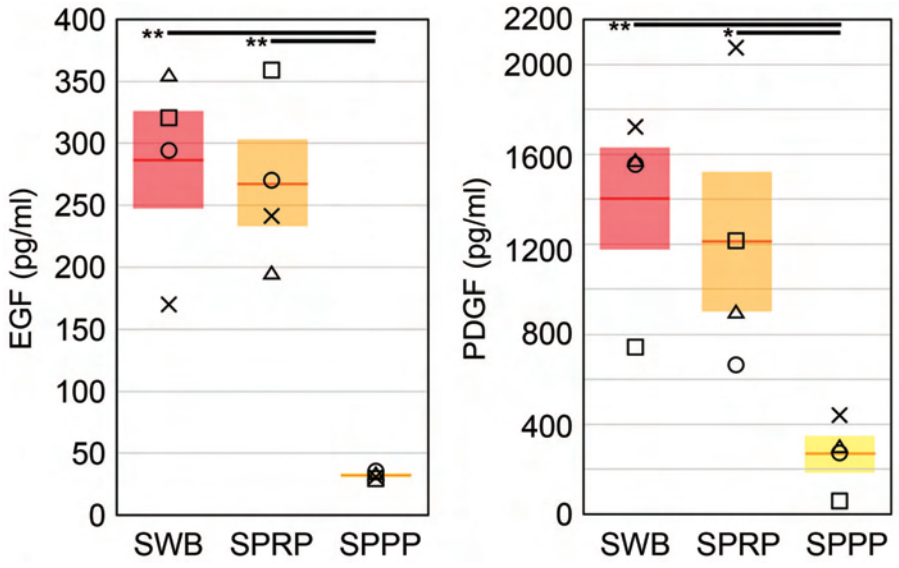

Fig. 4. Concentrations of platelet-derived cytokines in serum from whole blood, platelet-rich plasma, and platelet-poor plasma. Concentrations of two representative platelet-derived growth factors, EGF and PDGF, were measured in three types of serum prepared using various centrifugation conditions. Mean values are represented by *dark-colored horizontal bars*, whereas *light-colored boxes* indicate a range of SE. Values for each donor are indicated with the symbols listed in Table 1. Paired *t* tests were used for statistical analysis (*$p < 0.05$; **$p < 0.01$). *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma; ○, patient 1; △, patient 2; □, patient 3; ×, patient 4.

man dermal fibroblasts appeared in 4 to 7 days around the interstitial tissue fragments (after the initiation of outgrowth cultures) and became confluent after 2 to 3 weeks.

Preparation of Human Adipose-Derived Stem Stromal Cells

Informed consent was obtained from each participant before collection of lipoaspirates from body-contouring surgery, according to the institutional review board–approved protocol. Human adipose-derived stem/stromal cells were isolated from the samples and cultured as previously described.[22] In brief, the suctioned fat was digested with 0.075% collagenase in phosphate-buffered saline solution for 30 minutes with agitation at 37°C. Mature adipocytes and connective tissues were separated from pellets by centrifugation at 800 *g* for 10 minutes. Cell pellets were passed through a 100-μm mesh filter (Millipore, Bedford, Mass.) to remove debris and plated at a density of 5×10^6 nucleated cells/100-mm plastic dish. Cells were cultured in M199 medium containing 10% fetal bovine serum at 37°C under 5% carbon dioxide in a humidified incubator.

Preparation of Human Umbilical Vein Endothelial Cells

Before obtaining the placenta and umbilical cord samples, informed consent was obtained from each participant according to the protocol approved by the institutional review board. Isolation and culture of human umbilical vein endothelial cells was performed according to the method described by Jaffe et al.[23] Samples were collected immediately after delivery, separating the umbilical cord from the placenta by clipping both ends, and irrigated using 1% iodine/phosphate-buffered saline solution. To eliminate iodine, the intracelial space was rinsed using M199 medium and filled with 0.25% trypsin in phosphate-buffered saline. Both ends were again clipped, followed by incubation for 10 minutes at 37°C. Then, the intracelial space was rinsed using endothelial basal medium (Cambrex, Walkersville, Md.), and cells were collected. The cells were centrifuged at 450 *g* for 5 minutes, attached to 100-mm plastic dishes, and cultured with endothelial basal medium containing 2% fetal bovine serum.

Cell Proliferation Assay Using Culture Medium Containing Various Sera

Standard culture media were prepared (Dulbecco's Modified Eagle Medium for human dermal fibroblasts, M199 for human adipose-derived stem/stromal cells, and endothelial basal medium for human umbilical vein endothelial cells) containing fetal bovine serum, serum from whole blood, platelet-rich plasma, or platelet-poor plasma at concentrations of 0%, 5%, and 10% for human dermal fibroblasts and human adipose-derived stem/stromal cells, and 0%, 1%, and 2% for human umbilical vein endothelial cells, respectively. A total of 5×10^4 cells were plated in 60-mm dishes containing the prepared medium, and the medium was changed on the third and fifth days. Cells were counted on day 7 using a cell counter (NucleoCounter; Chemometec, Co., Allerod, Denmark). The average numbers were calculated from three different cultures for each cell type and culture condition.

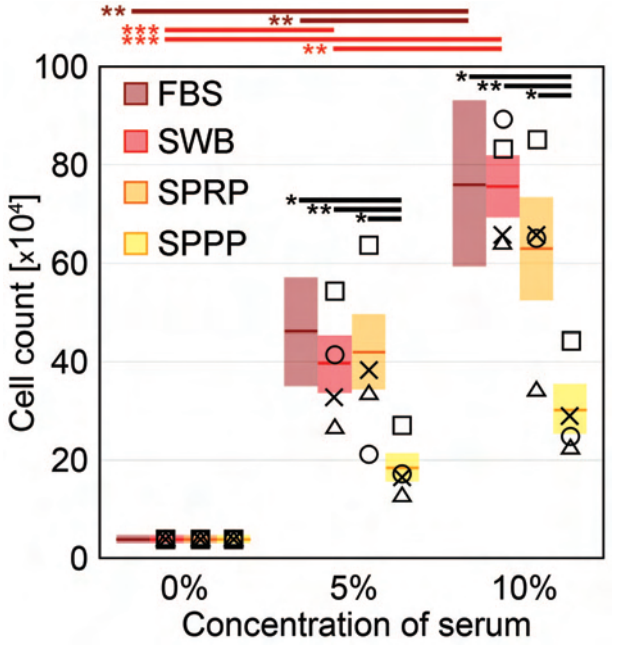

Fig. 5. Human-dermal fibroblast proliferation assay. Cell counts of human dermal fibroblasts on day 7 of culture with addition of fetal bovine serum, serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma at concentrations of 0%, 5%, and 10%. Mean values are represented by *dark-colored horizontal bars*, whereas *light-colored boxes* indicate a range of SE. Values for each donor are indicated with the symbols listed in Table 1. Paired *t* tests for human sera comparisons and unpaired *t* tests for fetal bovine serum and human sera comparisons were used for statistical analyses. *Horizontal bars* indicate statistical significance between different concentrations of each serum or between different types of serum at the same concentration (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *FBS*, fetal bovine serum; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma; ○, patient 1; △, patient 2; □, patient 3; ×, patient 4.

Statistical Analysis

Results were expressed as mean ± SE. To compare blood cell count and biochemical data for each sera, the values of serum from platelet-rich plasma and serum from platelet-poor plasma were described as a ratio to those of serum from whole blood. The unpaired *t* test was used to evaluate the differences in data between fetal bovine serum and human sera, and the paired *t* test was used for evaluations among three types of human sera because all three types were prepared from the blood of four identical donors. No correction was made for multiple comparisons. Statistical significance was defined as $p < 0.05$.

RESULTS

Blood Cell Count and Biochemical Analysis of Whole Blood, Platelet-Rich Plasma, and Platelet-Poor Plasma

The numbers of red blood cells, white blood cells, and platelets, and levels of total protein and albumin in whole blood at the time of blood collection were $428.0 \pm 23.7 \times 10^4$ cells/μl, 6100.0 ± 980.6 cells/μl, $25.0 \pm 1.5 \times 10^4$ cells/μl, 7.0 ± 0.4 g/dl, and 4.2 ± 0.4 g/dl, respectively. As shown in Figure 3, in platelet-rich plasma, 75.1 percent of platelets remained in comparison with whole blood, although red blood cells and white blood cells decreased to 0.6 percent and 7.1 percent, respectively. In platelet-poor plasma, the number of platelets decreased to 12.6 percent with a decrement of red blood cells and white blood cells to 0.35 percent and 2.2 percent, respectively. Despite these reductions in blood cell numbers, the values for total protein and albumin were relatively uniform. In addition, we identified no notable changes in electrolyte levels.

PDGF and EGF Concentrations in Serum from Whole Blood, Platelet-Rich Plasma, and Platelet-Poor Plasma

Concentrations of PDGF and EGF were analyzed quantitatively by enzyme-linked immunosorbent assay. As shown in Figure 4, in comparison with the values for serum from whole blood, serum from platelet-rich plasma contained 86.5 percent and 93.5 percent of PDGF and EGF respectively, whereas serum from platelet-poor plasma included only 19.1 percent and 11.2 percent of those growth factors, respectively. Serum from whole

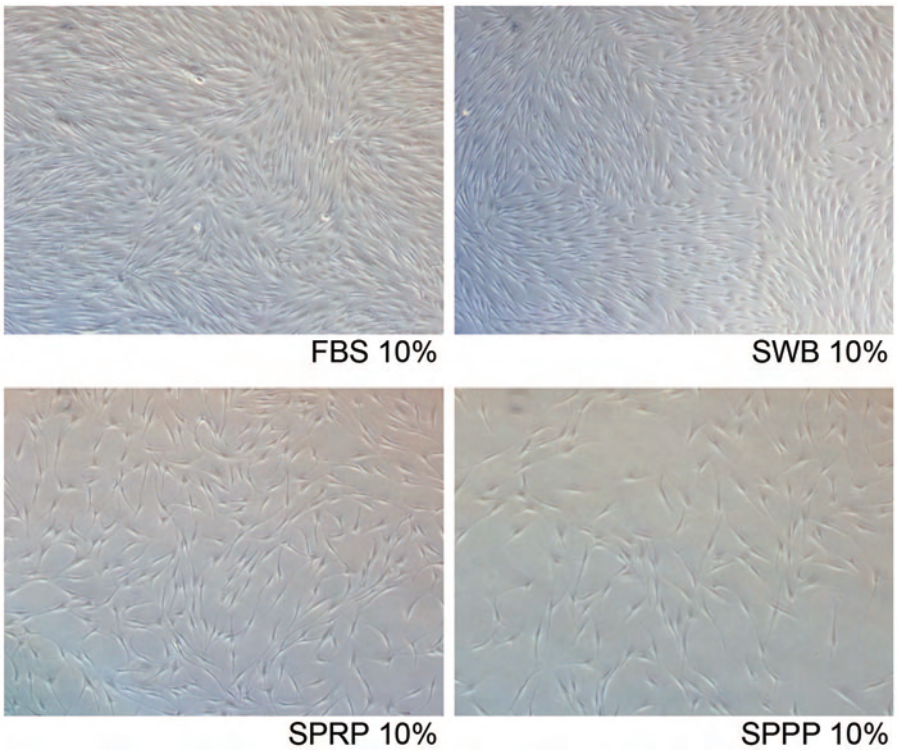

Fig. 6. Representative microscopic views of human dermal fibroblasts (day 7) cultured with 10% fetal bovine serum, serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma. *FBS*, fetal bovine serum; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma.

blood and platelet-rich plasma were consistently more abundant than serum from platelet-poor plasma in both platelet-derived cytokines in all four donors. A sample from the male donor contained a lower value for EGF and a higher value for PDGF compared with samples from the three female donors.

Effects of Serum from Whole Blood, Platelet-Rich Plasma, and Platelet-Poor Plasma on the Proliferation of Various Cell Types

The number of proliferated cells in the culture media with different serum types at various concentrations was compared with that obtained by culture with fetal bovine serum at the same concentration, using human dermal fibroblasts, human adipose-derived stem/stromal cells, and human umbilical vein endothelial cells. Although the degree differed among the cell types, human-originated sera were effective for the expansion of cell number by culture.

In the human dermal fibroblast culture, serum from whole blood and serum from platelet-rich plasma exhibited a high proliferative efficacy that was almost identical to that of fetal bovine serum, although cells cultured from serum from platelet-poor plasma showed a significantly lower degree of proliferation compared with other sera (Fig. 5). Although the degree of proliferative capacity of each type of serum differed among the donors, serum from whole blood and serum from platelet-rich plasma were consistently superior to serum from platelet-poor plasma in all four donors. Representative microscopic views of cultured human-dermal fibroblasts are shown in Figure 6.

In the human adipose-derived stem/stromal cell culture, although with the addition of serum from whole blood or platelet-rich plasma cell proliferation outcome was inferior to that for fetal bovine serum, the efficacy of cell proliferation was enhanced with increasing concentration of serum human products (Fig. 7). There was no significant difference among effects of serum from whole blood, serum from platelet-rich plasma, and serum from platelet-poor plasma. Representative microscopic views of cultured human adipose-derived stem/stromal cells are shown in Figure 8.

Proliferation of human umbilical vein endothelial cells with serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma did not differ significantly among the three types of human sera and was not as robust as that which occurred with addition of fetal bovine serum (Fig. 9). Representative microscopic views of cultured human umbilical vein endothelial cells are shown in Figure 10. No significant correlation was identified between proliferative activities of sera and age or sex of donors in any of the three human cell types.

DISCUSSION

Because specific gravity differs among various blood components, we can isolate each one by a specific centrifugation protocol; however, cell contamination cannot be avoided completely because of slight overlaps among these specific gravities. With our separation protocol, subtraction of red blood cells and white blood cells was sufficient in both platelet-rich plasma and platelet-poor

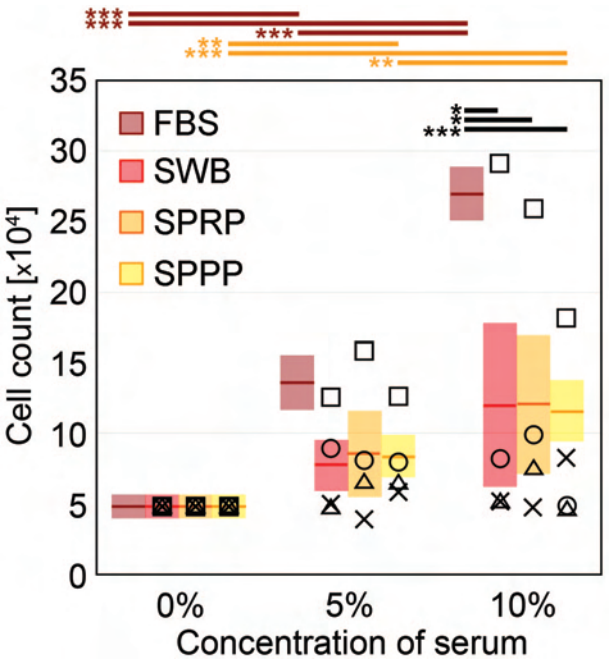

Fig. 7. Human adipose-derived stem/stromal cell proliferation assay. Cell numbers of human adipose-derived stem/stromal cells on day 7 cultured with addition of fetal bovine serum, serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma at concentrations of 0%, 5%, and 10%. Mean values are represented by *dark-colored horizontal bars*, whereas *light-colored boxes* indicate a range of SE. Values for each donor are indicated with the symbols listed in Table 1. Paired *t* tests for human sera comparisons and unpaired *t* tests for fetal bovine serum and human sera comparisons were used for statistical analyses. *Horizontal bars* indicate statistical significance between different concentrations of each serum or between different types of serum at the same concentration (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *FBS*, fetal bovine serum; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma; ○, patient 1; △, patient 2; □, patient 3; ×, patient 4.

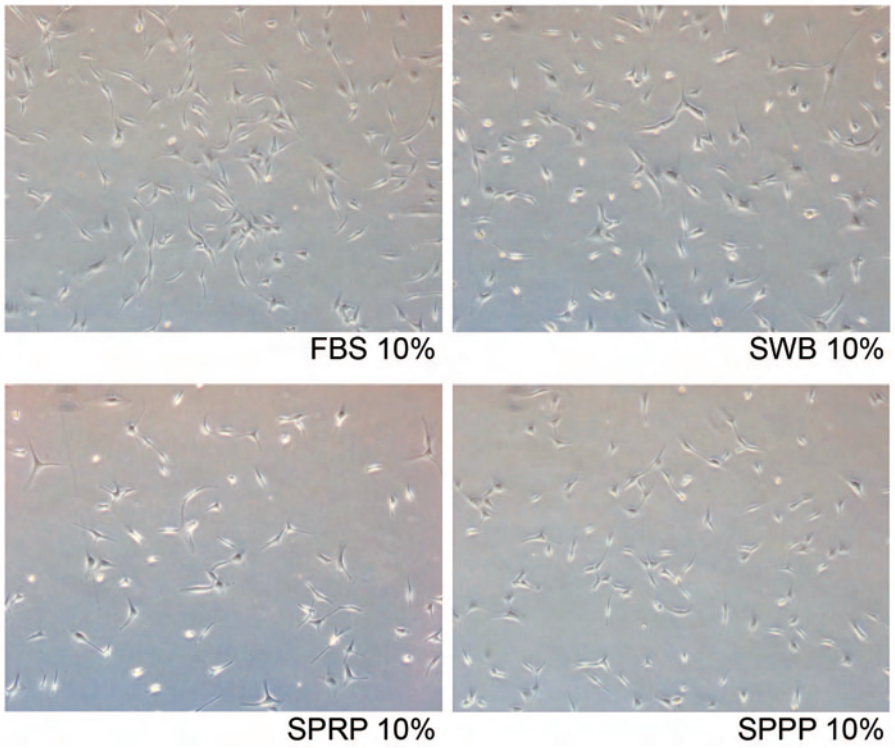

Fig. 8. Representative microscopic views of human adipose-derived stem/stromal cell (day 7) cultured with 10% fetal bovine serum, serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma. *FBS*, fetal bovine serum; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma.

plasma, and platelets were successfully preserved in platelet-rich plasma compared with platelet-poor plasma. Specifically regarding red blood cells, less than 1 percent of the original numbers in whole blood remained in platelet-rich plasma or platelet-poor plasma. An expected advantage in the future use of these component-level serum (or plasma) products in regenerative medicine lies primarily in the possibility of salvage use of red blood cells, conferring greater interest in serum from platelet-rich plasma and serum from platelet-poor plasma than in serum from whole blood.

Secretory proteins such as PDGF, EGF, transforming growth factor-β1, and vascular endothelial growth factor are stored in the α-granules of platelets and released by platelet activation by means of addition of thrombin[17,20] or adenosine diphosphate,[20] or by a freeze/thaw cycle.[11,16] Preparation and activation methods influence secretory protein concentrations.[18,21] Platelet activation with thrombin, which we used in this study, is considered to closely imitate the physiologic activation of platelets, ensuring the bioactivity of secreted growth factors.[6,24] In the present study, the concentration of total protein and albumin decreased slightly in the separation process of platelet-rich plasma and platelet-poor plasma from whole blood, but the concentrations of PDGF and EGF significantly decreased in proportion to the reduction in platelet count. Platelet-derived growth factors and platelet count were considered to be intimately associated, although the alteration was not linear.[15,16,19]

Serum from whole blood and serum from platelet-rich plasma showed a high proliferative effect on human dermal fibroblasts, an effect almost identical to that of fetal bovine serum, whereas human dermal fibroblasts cultured in serum from platelet-poor plasma showed a significantly lower degree of proliferation in all four donors involved in this study. Some platelet-originated growth factors, such as PDGF, are notable mitogens for human dermal fibroblasts.[19,25] The difference in human dermal fibroblast proliferation effects among serum from whole blood, platelet-rich plasma, and platelet-poor plasma may arise primarily from differences in concentrations of the platelet-originated growth factors.

In human adipose-derived stem/stromal cell culture, although cell proliferation was generally

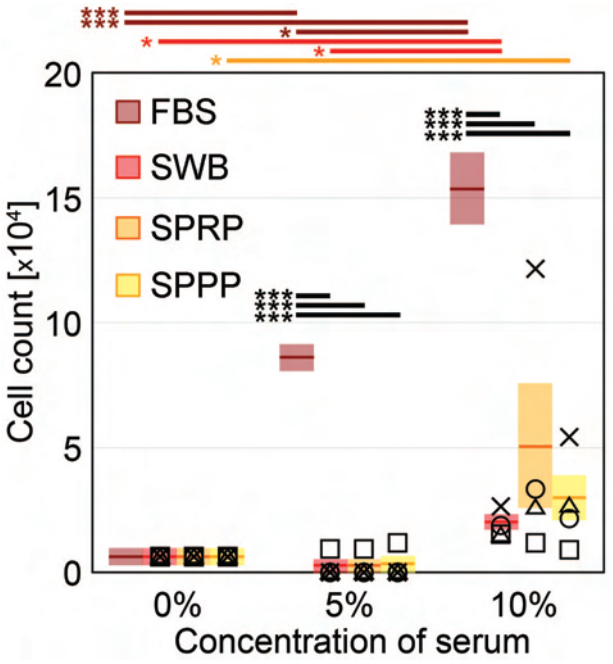

Fig. 9. Human umbilical vein endothelial cell proliferation assay. Numbers of human umbilical vein endothelial cells on day 7 cultured with fetal bovine serum, serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma at concentrations of 0%, 1%, and 2%. Mean values are represented by *dark-colored horizontal bars*, whereas *light-colored boxes* indicate a range of SE. Values for each donor are indicated with the symbols listed in Table 1. Paired *t* tests for human sera comparisons and unpaired *t* tests for fetal bovine serum and human sera comparisons were used for statistical analyses. *Horizontal bars* indicate statistical significance between different concentrations of each serum or between different types of serum at the same concentration (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *FBS*, fetal bovine serum; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma; ○, patient 1; △, patient 2; □, patient 3; ×, patient 4.

enhanced depending on the concentrations of the three human serum preparations, cell proliferation outcome differed greatly among the donors and was generally inferior to that achieved with fetal bovine serum. Our results using human sera obtained from the same four donors are inconsistent with the previous finding of Kocaoemer et al.[6] that the proliferative efficacies of pooled human antibody serum (corresponding to serum from whole blood in our study) and thrombin-activated platelet-rich plasma (corresponding to serum from platelet-rich plasma in our study) surpassed that of fetal bovine serum. Platelets do not provide some major growth factors, such as basic fibroblast growth factor (bFGF), keratinocyte growth factor, and hepatocyte growth factor.[7,26] This may be the reason that human platelet-originated growth factors are not sufficient for expansion of human adipose-derived stem/stromal cells; fetal bovine serum may contain ingredients more influential for human adipose-derived stem/stromal cell proliferation, such as bFGF.

For manipulating stem cells in regenerative medicine, differentiation capacity and proliferation capacity should be considered, and an optimal culture additive differs according to the purpose of the culture. Because PDGF is known to be a potent inhibitor of adipogenic differentiation of human adipose-derived stem/stromal cells, serum from platelet-poor plasma with a selective addition of recombinant growth factors such as bFGF and/or EGF may be preferable to serum from whole blood in human adipose-derived stem/stromal cell culture for adipose tissue engineering.[14,27] In our study, differentiation capacity after cell expansion was not assessed because of the volume limitation of the samples.

In human umbilical vein endothelial cell culture, cell growth with either fetal bovine serum or human serum preparations was inferior to that in a specific endothelial growth medium (data not shown), probably because growth factors such as bFGF and vascular endothelial growth factor that are not sufficiently present in serum are critical factors for human umbilical vein endothelial cell proliferation. The results of all human serum preparations were significantly worse than those obtained with fetal bovine serum, although the three human serum preparations showed no significant differences among one another. A supplemental use of angiogenic growth factors may enhance the proliferative effect of serum products on human umbilical vein endothelial cells.

CONCLUSIONS

To our knowledge, this study is the first to compare different human serum preparations as an additive of cell culture, using blood samples obtained from identical donors. We found that serum from whole blood and serum from platelet-rich plasma are superior to serum from platelet-poor plasma as substitutes for animal-derived serum in culture expansion of human dermal fibroblasts. Platelet-derived ingredients, however, are considered nonessential or insufficient for enhanced proliferation of human adipose-derived stem/stromal cells and human umbilical vein endothelial cells. Although autologous or human-derived serum preparations may be of great use in cell-based therapies in the future, this usefulness depends strongly on the target cell species and the

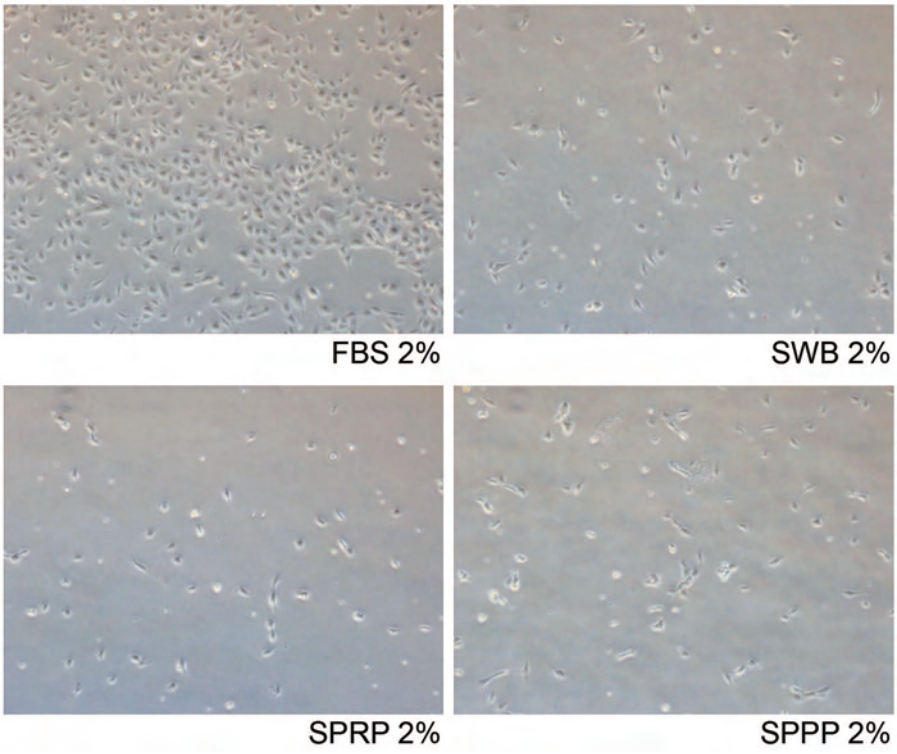

Fig. 10. Representative microscopic views of human umbilical vein endothelial cells (day 7) cultured with 10% fetal bovine serum, serum from whole blood, serum from platelet-rich plasma, or serum from platelet-poor plasma. *FBS*, fetal bovine serum; *SWB*, serum from whole blood; *SPRP*, serum from platelet-rich plasma; *SPPP*, serum from platelet-poor plasma.

purpose of the cell culture. Future studies should focus on establishing the optimal indications of each human serum preparation.

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