Characterization of wound drainage fluids as a source of soluble factors associated with wound healing: comparison with platelet-rich plasma and potential use in cell culture

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ABSTRACT

Wound fluids, human serum from platelet-poor and platelet-rich plasma (SPPP and SPRP), contain various soluble factors involved in cell growth and proliferation. Levels of cytokines, chemokines, and matrix metalloproteinases (MMPs) in drainage fluids (DFs) harvested from subcutaneous wounds, punctured fluids (PF) from seroma, and SPPP were measured. SPPP and SPRP from four healthy volunteers were also subjected to the analysis. Biochemical profiles of DF reflected the sequential stages of wound healing. Early-phase DF contained high concentrations of basic fibroblast growth factor and platelet-derived growth factor and EGF. The levels of keratinocyte growth factor, interleukin-6, and MMP-8 in DF peaked on days 2–3, while vascular endothelial growth factor, hepatocyte growth factor, interleukin-8, and MMP-1 increased over time during days 0–6. Punctured fluids contained high levels of TGF-β1, keratinocyte growth factor, vascular endothelial growth factor, hepatocyte growth factor, and MMP-1. Experiments using human adipose-derived stem cells and dermal fibroblasts cultured in media containing various concentrations of DF and fetal bovine serum suggested that for some cell types, DF-contained growth factors that are not obtained from SPRP could be used to supplement or substitute for serum in culture media. SPRP and DF are economical ready-made mixtures of serum and autologous soluble factors, and may be differentially useful for regenerative therapies.

One of the requirements for successful cell-based therapy is the delivery of stem cells to target tissues after manipulations such as the expansion stem cell cultures or commitment of stem cells to a specific lineage. However, due to safety considerations such as transmission of viral or prion-related disease, the use of animal-derived products such as serum, tissue extracts, and enzymes in these manipulations is undesirable. Hence, several studies have examined the use of human-derived substitutes: Attempts were made to use human autoserum as a replacement for fetal bovine serum (FBS),[1] although its volume available is limited. Patient-derived fibrin glue (thrombin and fibrinogen) and platelet-rich plasma (PRP) have also been used for cell culture or in clinical trials for enhancing tissue regeneration.[2] Other studies have shown that growth factors derived from platelets can be used to stimulate cell proliferation,[3–5] but platelets cannot provide some other major growth factors.[6]

The growth factor b-fibroblast growth factor (b-FGF), which is an important endogenous stimulator of angiogenesis[7] and cell proliferation,[8] is released from surrounding wounded tissues during an early phase of wound healing.[9,10] Cellular b-FGF is released by the lysis of epidermal cells,[11] fibroblasts,[11] and endothelial cells (EC)[12] around the wound, and b-FGF bound up in the extracellular matrix (ECM) is released by the action of various wound proteases.[13,14] Keratinocyte growth factor (KGF) is expressed in the dermis during wound healing,[15] and stimulates wound reepithelialization.[16] Hepatocyte growth factor (HGF) was independently discovered as a powerful mitogen for hepatocytes and as a stimulator of dissociation

ASC	Adipose derived stromal cell
b-FGF	Basic fibroblast growth factor
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
HGF	Hepatocyte growth factor
IGF-1	Insulin-like growth factor
KGF	Keratinocyte growth factor
PDGF	Platelet-derived growth factor
PRP	Platelet-rich plasma
SPPP	Human serum from platelet-poor plasma
SPRP	Human serum from platelet-rich plasma
TGF-β	Transforming growth factor-β
VEGF	Vascular endothelial growth factor

of epithelial cells.[17] HGF-producing cells are found among those of mesenchymal origin, and HGF stimulates cell proliferation, cell migration,[18] and the production of matrix metalloproteinase (MMP)[19] in keratinocytes.

Various growth factors directly or indirectly control phenomena accompanying wound-healing inflammation, remodeling, and regeneration,[17] and can be detected in cutaneous wound fluids,[20–22] or in wound fluids obtained through surgical suction drains.[23–25] The fluid composition and the concentration of growth factors in the wound fluids change as healing progresses, and thus the fluids reflects the sequential wound-healing phenomena. However, there is currently only limited information pertaining to the characterization of surgical drainage fluid (DF) with regard to growth factors and other soluble factors. Although a few previous reports have suggested that wound fluids have mitogenic and chemotactic effects,[8,26,27] there has been limited information beyond that. Also, although it may be clinically feasible to use wound fluids for cell-based regenerative therapies, protocols for use of wound fluids have yet to be optimized.

The present study focuses on the characterization of subcutaneous wound fluids obtained through surgical suction drains. Such fluids can be aseptically harvested with minimal morbidity: for example, adipose-derived stem cells can be isolated from liposuction aspirates and subcutaneous wound fluids can be simultaneously obtained by leaving a suction drainage tube in the subcutaneous cavity in the same surgery. We sequentially collected surgical DFs from the subcutaneous space after plastic surgery, and characterized the fluids by examining wound healing-associated soluble factors such as electrolytes, cytokines, chemokines, and MMPs. We compared these characterization profiles with those of platelet-poor (PPP) and PRP, which can also be easily obtained from patients, and all three types of fluids were assessed for their potential utility in cell culture.

MATERIALS AND METHODS

Collection and preparation of human sera from PRP and PPP

Human PRP and PPP were prepared from four healthy volunteers. Collection of human-derived fluids (serum, DF, and punctured fluid [PF] samples) was conducted under protocols as approved by the ethical committee of University of Tokyo School of Medicine and in accordance with ethical treatment of human subjects. Blood was drawn into two 200 mL blood bags containing 0.327% citric acid, 2.63% sodium citrate, 0.0275% adenine, 0.251% sodium dihydrogen phosphate, and 2.9% D-glucose solution (blood bag CPD-adenine®; Terumo, Tokyo, Japan). To isolate PRP, bags were centrifuged at 200×*g* for 10 minutes in a large-capacity refrigerated centrifuge (Kubota 9810; Kubota Co., Tokyo, Japan), and to isolate PPP the bags were centrifuged at 5,000×*g* for 5 minutes; in both instance, the supernatant was harvested. To obtain serum from PRP (designated SPRP) and from PPP (designated SPPP), 100 mL of PRP or PPP was drawn into a flask and 200 U of thrombin was added. The flask was agitated for 60 minutes at 37 °C and then incubated overnight at 4 °C, after which the liquid component was drawn into a 50 mL tube, centrifuged at 2,000×*g* for 10 minutes, and the supernatants were obtained as for SPRP and SPPP. The serum samples were frozen at −80 °C and thawed at 37 °C before analysis.

Collection and preparation of suction DF samples

We collected DFs and PFs from subcutaneous wounds from 15 patients, who underwent liposuction and abdominoplasty (seven patients), liposuction (five patients), and breast augmentation (three patients) at the University of Tokyo Hospital. Two to three weeks before surgery, 400 mL of blood was harvested from 10 of the 15 patients. The blood was separated by centrifugation into concentrated red blood cells and PPP; the cells were stored in preparation for autotransfusion, and the PPP was processed to obtain SPPP, using the previously described method. Suction drains (J-vac drainage system; Johnson & Johnson, Cornelia, GA) were subcutaneously inserted into operative wounds during surgery, after which wound fluid was continuously suctioned at 40–60 mmHg and aseptically collected in the storage bag of the suction system. The wound fluids were centrifuged at 2,000×*g* for 30 minutes, and the supernatant fluids were frozen at −80 °C. The frozen samples were thawed at 37 °C before analysis. DF samples ($N=52$) obtained from 12 patients whose wounds exhibited normal healing (NH group) and PFs ($n=4$) obtained from three patients who had subcutaneous seroma formation (SF group) were used in this study. Nineteen samples harvested on day 0 or 1 from 12 patients of the NH group were referred to as *Drainage Fluid-Early* (*DF-E*), while nine samples harvested on day 5 or 6 from seven of the 12 patients of NH group were referred to as *Drainage Fluid-Late* (*DF-L*). PF samples were harvested by puncturing the subcutaneous seroma on day 14 or later, and were included in our analyses as *Punctured fluids* (*PF*).

Biochemical analysis of serum and DF constituents

From each of three patients, we obtained a set of a preoperative serum samples and seven sequential DFs (days 0–6). These samples (a total of eight from each person) were subjected to biochemical analyses for total protein, albumin, sodium, potassium, chloride, calcium, and iron. Analysis was performed by SRL Inc. (Tachikawa, Japan), a commercial analysis service.

Quantitative assays for cytokines, chemokines, and MMPs associated with wound healing

The concentrations of various cytokine growth factors (platelet-derived growth factor [PDGF-BB], epidermal growth factor [EGF], transforming growth factor-β1 [TGF-β1], b-FGF, vascular endothelial growth factor [VEGF], HGF, KGF, and insulin-like growth factor-1 [IGF-1]) and chemokines (interleukin-6 [IL-6] and IL-8) in DF samples, PFs, PRP, and PPP were assayed using anti-human enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®; R&D Systems Inc., Minneapolis, MN), according to the manufacturer's instructions. The levels of

immunoreactive cytokines as reported by the ELISA assay were measured at 450 nm by a microplate reader (Model 550; Biorad Laboratories, Hercules, CA), and a standard curve was generated to determine the growth factor concentrations (pg/mL). The levels of MMP-1, MMP-8, and MMP-13 in DFs were also measured using anti-human ELISA kits (Biotrak ELISA System; Amersham Biosciences, Piscataway, NJ).

Primary cell culture

Adipose-derived stromal cells (ASCs) were isolated from human lipoaspirates and cultured as described previously.[28] Briefly, the suctioned fat was digested with 0.075% collagenase in phosphate-buffered saline (PBS) for 30 minutes with agitation at 37 °C. Mature adipocytes and connective tissues were separated from pellets by centrifugation (800× *g*, 10 minutes). Cell pellets were resuspended in erythrocyte lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA), incubated for 5 minutes at room temperature, resuspended again and passed through a 100 µm mesh filter (Millipore, Billerica, MA), and then plated at a density of 5×10^6 nucleated cells/100 mm plastic dish. Cells were cultured in M-199 medium containing 10% FBS at 37 °C under 5% CO_2 in a humidified incubator.

Human dermal fibroblasts were isolated from normal skin samples obtained from plastic surgery. The skin samples were cut into pieces of approximately 3×3 mm and treated with 0.25% trypsin in PBS for 24 hour at 4 °C. After removal of the epidermis, the connective tissue fragments were attached to 100 mm plastic dishes and cultured with DMEM containing 10% FBS. Primary fibroblasts appeared 4–7 days after the initiation of outgrowth cultures and became confluent after 2–3 weeks.

Cell proliferation assay using culture medium containing DFs

Culture media (M199 for ASCs and Dulbecco's modified Eagle's medium [DMEM] for dermal fibroblasts) containing FBS and/or DFs (DF-E or DF-L) were prepared at various concentrations. The DF samples were sterilized using a 0.22 µm filter (MILLEX GV Filter Unit, Millipore) before use. 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. The cell numbers were counted on the seventh day using a NucleoCounter (Chemometec, Allerod, Denmark), and the average numbers were calculated from three different cultures of the cell types for each condition. The averages were normalized by calculating a ratio of cell numbers grown in each condition to cell numbers grown under the standard culture conditions (5% FBS without DF).

Statistics

The Kruskal–Wallis *H*-test with post hoc comparisons was used to compare the concentrations of cytokines, chemokines, and MMP among drainage fluids (DF-E and DF-L), PFs, and SPPP. The Wilcoxon matched-pair *t* test was used for comparison between SPRP and SPPP obtained from the same four volunteers, and also to compare the cell numbers in each culture condition with that of control. $p < 0.05$ was considered to be significant. The statistical analyses were reviewed by an appropriately knowledgeable individual.

RESULTS

Comparison of cytokine concentrations in SPRP and SPPP

The concentrations of various cytokine growth factors in SPRP and SPPP harvested from four volunteers are shown in Figure 1. The concentrations of EGF, PDGF, TGF-β, VEGF, and HGF were significantly higher in SPRP than in SPPP. The difference between the two sera was markedly seen in concentrations of EGF and PDGF. IGF-1 was similarly present in both SPRP and SPPP, while bFGF and KGF were only minimally detected in both. PDGF detected in SPPP suggested a small amount of platelet contamination in SPPP.

Biochemical profiles of DFs and blood serum

The concentrations of total protein and albumin in DFs on day 0 were about 50% of those in preoperative serum, and both total protein and albumin gradually decreased to about 30% of that found in serum by day 6 (Figure 2). The concentrations of Na^+, K^+, Cl^-, Ca^{++}, and Fe^{++} in DFs were similar to those in the serum and did not significantly change with time. The concentration of Ca^{++} in DFs was about 60–70% of that in the serum and changed very little from days 0 to 6. The concentration of Fe^{++} in DF was extremely variable among different patients due to variations in an individual's hemorrhage volume, but in general was substantially greater than that in the serum, and tended to decrease slightly with time.

Cytokine concentrations in DFs and SPPP

The daily sequential changes in various cytokine growth factors in DFs from six (VEGF, KGF) or seven (all other cytokines) patients are shown in Figure 3, and the data of DF-E (n=19), DF-L (n=9), PF (n=4), and SPPP (n=10)

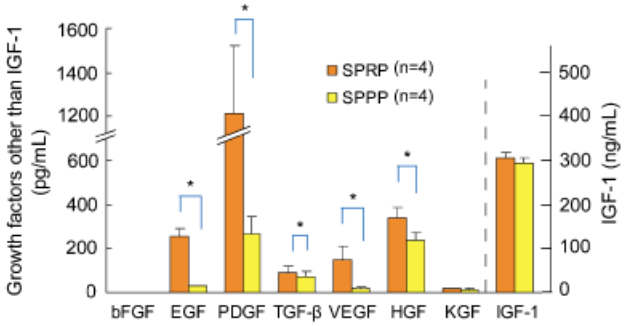

Figure 1. Comparison of cytokine concentrations in serum from platelet-rich plasma (SPRP) and serum from platelet-poor plasma (SPPP). SPRP and SPPP were collected from four volunteers. Cytokine concentrations were measured in SPRP and SPPP. Values represent means ± SE. *$p < 0.05$ (blue lines).

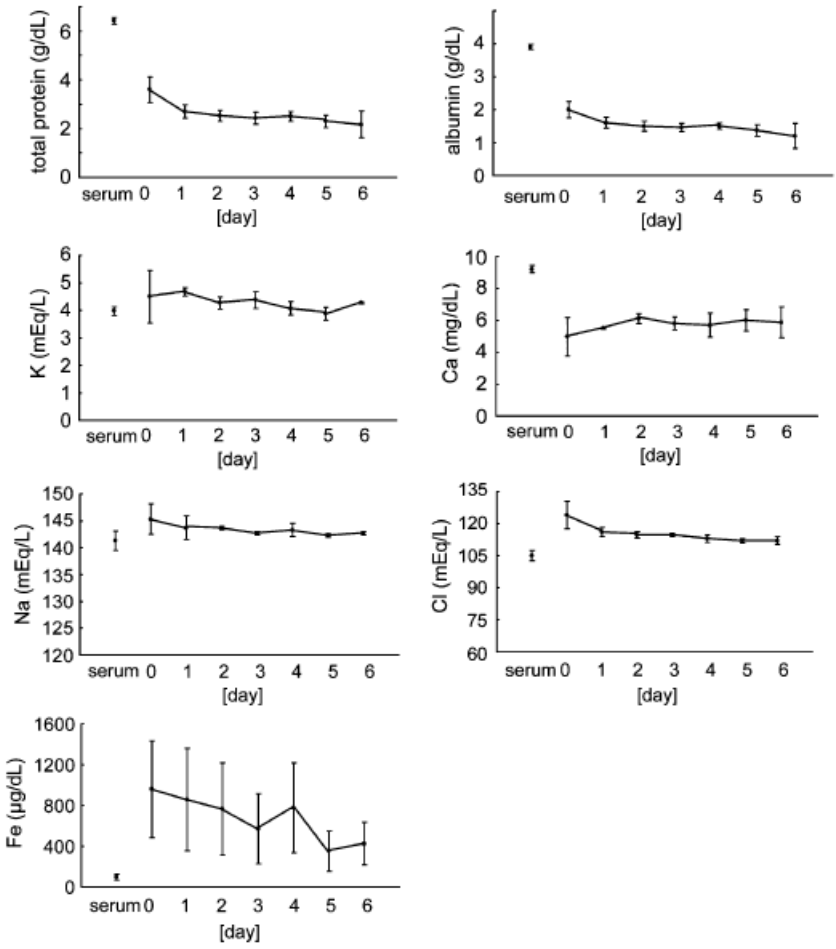

Figure 2. Biochemical profiles of preoperative serum and of drainage fluids (DFs) on days 0–6. Preoperative serum and DFs were collected from the same three patients; DFs were collected on days 0–6, where day 0 represents the day that the surgery was performed. The preoperative serum value is indicated to the left on the *x*-axis and is labeled ''serum''; the numbers 0–6 to the right represent day 0–6 time points of DF collection. Values represent means ± SE.

from patients are summarized in Figure 4. The concentrations of b-FGF, EGF, PDGF, and TGF-β were much higher in DF-E than in DF-L or SPPP. SPPP contained much lower concentrations of VEGF, HGF, and KGF compared with DF-E, DF-L, and PF. PF contained high levels of TGF-β, VEGF, HGF, and KGF, while the concentrations of b-FGF, EGF, and PDGF were sufficiently low in PF that they could not be detected.

KGF concentrations peaked around day 3 and then began to decrease, in contrast to VEGF and HGF concentrations, which steadily increased with successive postoperative days. IGF-1 did not change significantly with postoperative time, and the concentration of IGF-1 in DF-E and DF-L was significantly lower (by approximately 50%) compared with SPPP.

Chemokine and MMP concentrations in DFs, SPRP, and SPPP

Daily sequential changes in the IL-6 and IL-8 chemokines and in MMPs (collagenases) were tracked in DFs from seven patients, and the summarized data are shown in Figures 5 and 6. IL-6 was present at high concentrations in DF-E and decreased gradually in successive postoperative days, while IL-8 increased gradually. DF-E contained twice as much IL-6 as was found in DF-L, while DF-L contained more IL-8 than DF-E. Neither IL-6 nor IL-8 was detected in SPPP. PF contained both chemokines, although there were no significant differences in PF chemokine levels vs. chemokine levels in either DF-E or DF-L (Figure 5).

In DFs, MMP-8 was present in much higher amounts as compared with MMP-1 and MMP-13 (Figure 6). MMP-8 increased in the early phase of wound healing, peaked on days 2–3, and then gradually decreased with time.

Cell proliferation assays using culture medium including the DFs

In medium that had not been supplemented with DFs, ASCs proliferated in a dose-dependent manner with regard to the concentration of FBS; the dose-dependent relationship was valid up to 10% FBS (Figure 7A). When ASCs were grown in media containing 5% FBS, cell proliferation was significantly enhanced by the addition of DF-E or DF-L at concentrations of $\geq$1%. The increase in proliferation was dose-dependent with respect to the concentration of DF-E or DF-L. When 5% FBS and 5% DF-E were added

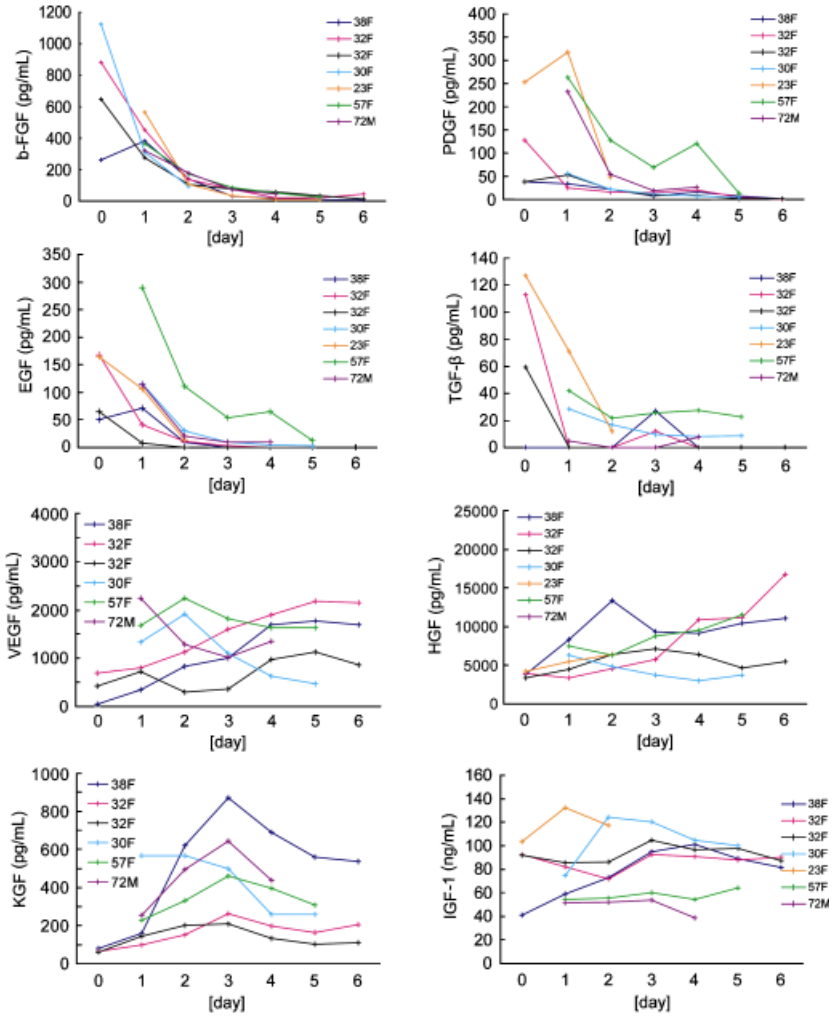

Figure 3. Daily changes in cytokine concentrations in drainage fluids (DFs). DFs were collected on days 0–6 from patients in the normal healing (NH) group, and cytokine concentrations were examined by enzyme-linked immunosorbent assay. In some patients, DFs were not obtained on all the designated days. For vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF), data were derived from six patients; for all other cytokines, data were derived from seven patients. Each line shows data from one patient (e.g., 38F means 38-year-old female).

to the medium, the cell count increased to over five times that of the control (5% FBS alone), and was twice as high as the cell count for media containing 10% FBS alone. In medium lacking FBS, the addition of DFs significantly enhanced ASC proliferation but were less effective compared with the same concentrations of FBS. We also examined dermal fibroblasts, which proliferated in a dose-dependent manner with respect to FBS up to concentrations of 10% FBS (Figure 7B). Although the proliferation of dermal fibroblasts was enhanced by DFs in the absence or presence (5%) of FBS, enhancement of proliferation by the addition of DFs was moderate. Based on a comparison between 5% FBS+5% DF-E (or DF-L) and 10% FBS, DFs showed no additional exhibited no value as an additive to FBS in terms of expansion of dermal fibroblasts.

DISCUSSION

Biochemical analysis of DFs

The results from the analysis of the biochemical composition of DFs in this study were similar to those of a previous study analyzing biochemical profiles of DFs after axillary dissection.[29] Our analysis showed that the concentrations of Na^+, K^+, and Cl^- in DFs were similar to those in the plasma, while concentrations of Ca^{++}, total protein, and albumin in DFs were $\sim$60–80% lower than those in plasma. The concentration of Fe^{++} in DF was generally higher than in the plasma but was variable (depending on the hemorrhage volume) and decreased over time.

Extracellular fluid volume, which makes up approximately 20% of the body weight, is composed of 5% plasma (or intravascular fluid) and 15% interstitial fluid. The concentrations of Na^+, K^+, and Cl^- in interstitial fluid are similar to those in plasma, while the total protein concentration of interstitial fluid is less than a third of that of plasma.[30] It is therefore likely that our DFs consisted primarily of interstitial fluid, with plasma composing the remaining $\sim$20–40% of the total volume. However, DFs are not simply a mixture of plasma and interstitial fluids because unlike these fluids, they also contain several other types of proteins, including various cytokine growth factors and chemokines.

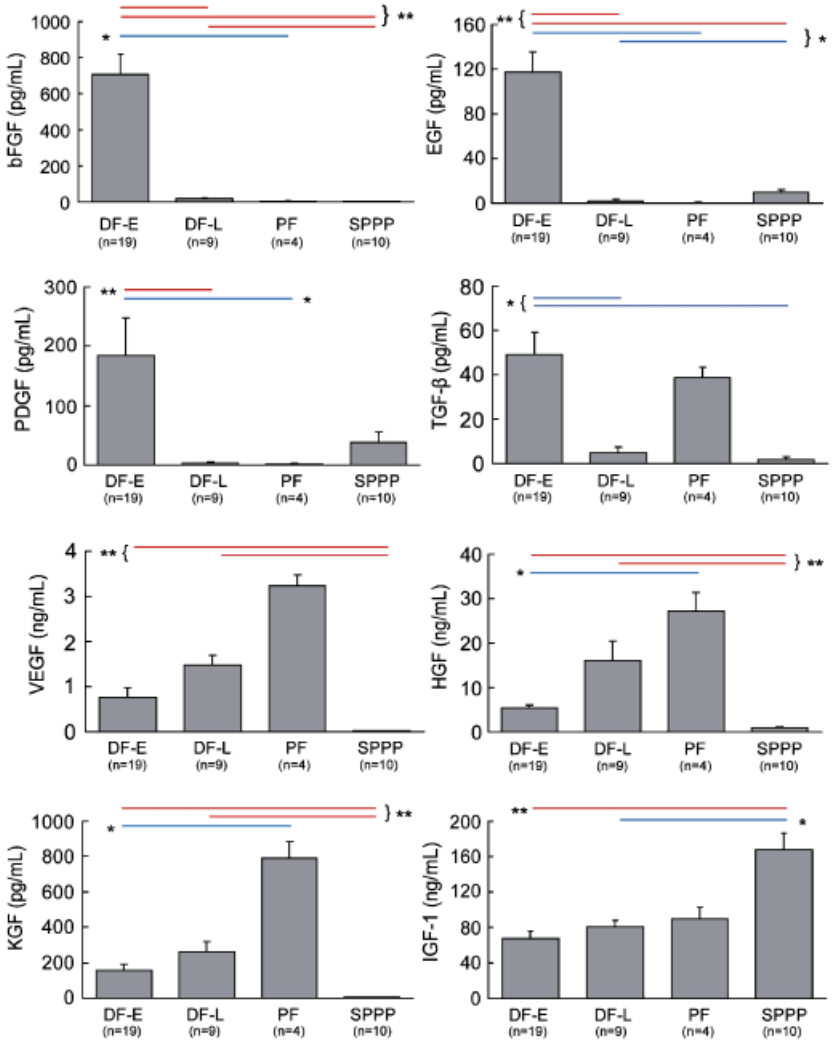

Figure 4. Comparison of cytokine concentrations in drainage fluid-early (DF-E), drainage fluid-late (DF-L), punctured fluid (PF), and serum from platelet-poor plasma (SPPP). Cytokine concentrations were measured in DF samples from day 0 or 1 (DF-E), DF from day 5 or 6 (DF-L), punctured seroma fluid samples from days 14+ (PF), and SPPP. Values represent means ± SE. $*p < 0.05$ (blue lines), $**p < 0.01$ (red lines).

Cytokine growth factor profiles in DFs

The sequential changes in cytokine profiles in DFs shown in this study clearly reflected the successive activities of various cells and the sequential phenomena involved in the wound-healing process (Figure 8). In the early phase (postoperative days 0–1) of wound healing, b-FGF, PDGF, EGF, and TGF-β1 were present at high concentrations, and the levels subsequently decreased acutely in the next stage of wound healing. Because b-FGF is known to be primarily derived from injured tissue or from cells infiltrating into wounds at early stages, tissue-bound b-FGF may be released after injury by several mechanisms, including cell lysis and cell injury.[11,12,31] PDGF, EGF, and TGF-β1 were detected at higher amounts in SPRP than in DF-E, suggesting that these growth factors were mainly supplied by dying, lytic platelets in the early phase of wound healing.

In the second phase of wound healing (postoperative days 2–4), KGF concentrations peaked, and those of VEGF and HGF increased slightly. Later, in the third phase, (days 5–6) VEGF and HGF concentrations gradually increased to peak levels. Because these growth factors were present at only very low levels on days 0–1, it is possible that these increases resulted from their release from the cells that migrated to the wound site after day 1. KGF, VEGF, and HGF are thought to have roles primarily in granulation, angiogenesis, and epithelialization.[32–34] KGF is known to be released mainly from fibroblasts and T cells,[35,36] VEGF from keratinocytes and macrophages,[37,38] and HGF from mesenchymal cells such as dermal fibroblasts.[18]

PFs from subcutaneous seroma contained higher concentrations of growth factors seen in later phases such as VEGF, HGF, and KGF, but TGF-β1, which is seen in the early phase, was also abundant in seroma fluid. This finding may be based on different sources of the early-phase growth factors: PDGF and EGF are mostly derived from platelet, whereas TGF-β1 is supplied not only from platelets but also from various sources. The initial production

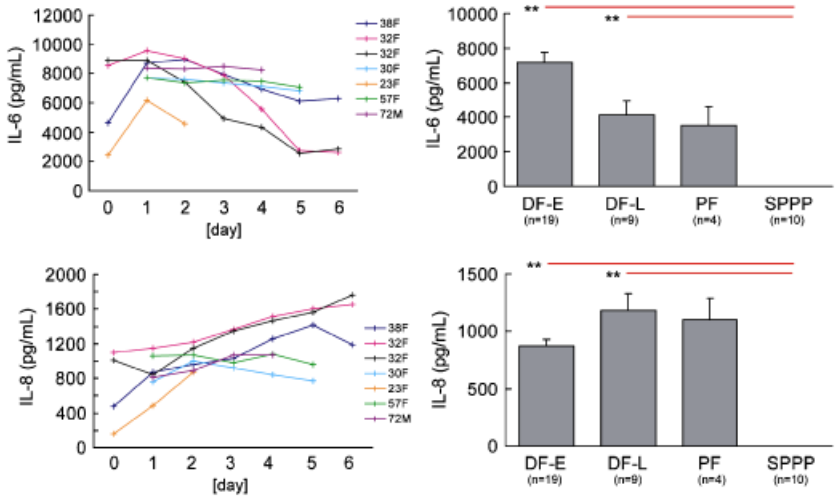

Figure 5. Changes in interleukin-6 (IL-6) and IL-8 concentrations in drainage fluid-early (DF-E), drainage fluid-late (DF-L), punctured fluid (PF), and serum from platelet-poor plasma (SPPP). The concentration of IL-6 and IL-8 was measured by enzyme-linked immunosorbent assay in drainage fluids collected on days 0–6 and was similarly measured in PF and SPPP. Data of daily changes from seven patients are shown in the left figures, in which each line shows data from one patient (e.g., 38F means 38-year-old female). Values in the right graphs represent means ± SE. $^{**}p < 0.01$ (red lines).

of active TGf-β1 from platelets serves as a chemoattractant for neutrophils, macrophages, and fibroblasts, and these cells further enhance TGF-β1 production.[17]

Chemokine and MMP profiles in DFs

In our study, IL-6, a major mediator of the host response to tissue injury,[39] was present in DF-E at about 7,000 pg/mL and gradually decreased afterwards. Cells that appeared in the wound area in each phase seemed to be major sources of IL-6; neutrophils were present in the early phase and macrophages and lymphocytes were present in the later phases. In the case of IL-8, the concentration gradually increased up to day 6, and so it is probable that the fibroblasts present in the second wave of cell migration to the wound produced significant amounts of IL-8, as was previously suggested in a study of fetal wound healing.[40]

Proinflammatory cytokines/chemokines directly stimulate the synthesis of the collagen-degrading MMPs and also inhibit the synthesis of tissue inhibitors of metalloproteinase

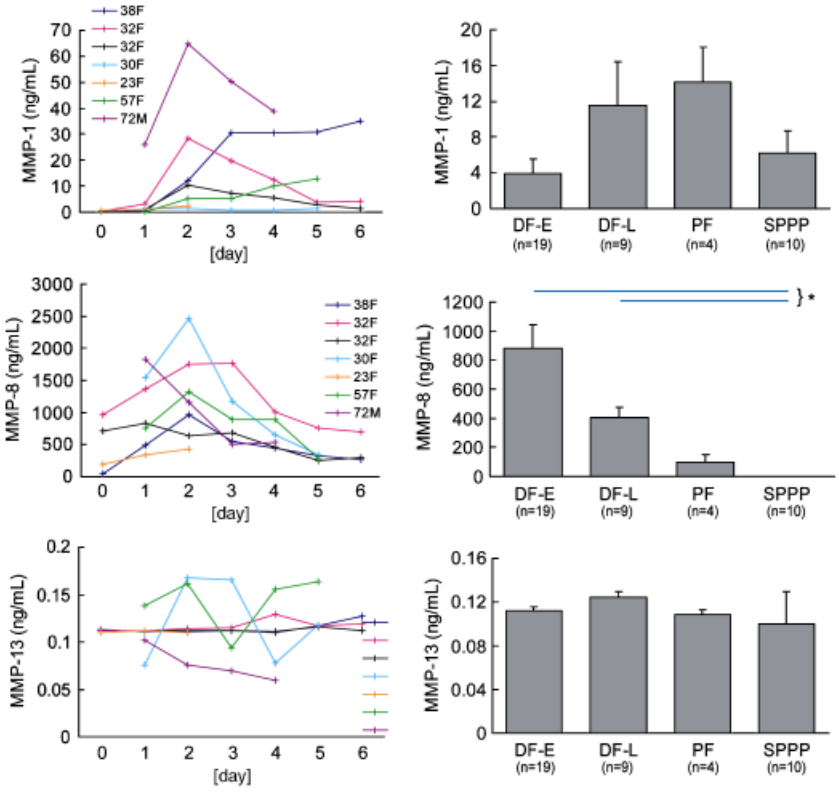

Figure 6. Changes in concentrations of matrix metalloproteinase-1 (MMP-1), MMP-8, and MMP-13 in drainage fluid-early (DF-E), drainage fluid-late (DF-L), punctured fluid (PF), and serum from platelet-poor plasma (SPPP). The concentration of MMP-1, MMP-8, and MMP-13 were measured by enzyme-linked immunosorbent assay in drainage fluid collected on days 0–6 and in seroma PF, SPPP, and serum from platelet-rich plasma (SPRP). Data of daily changes from seven patients are shown in the left figures, in which each line shows data from one patient (e.g., 38F means 38-year-old female). Values in the right graphs represent means ± SE. $^{*}p < 0.05$ (blue lines).

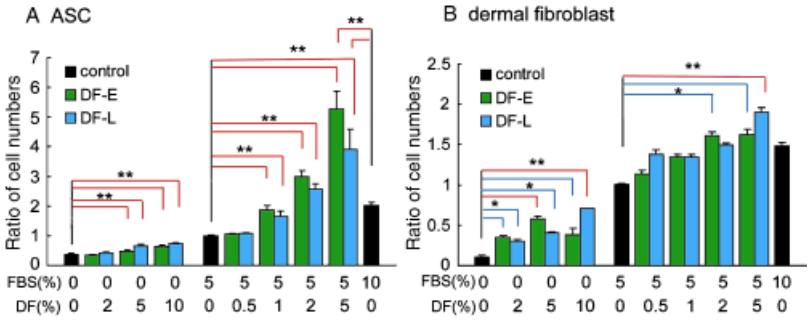

Figure 7. Proliferation of adipose tissue-derived stromal cells (ASCs) and dermal fibroblasts. ASCs (A) and dermal fibroblasts (B) were cultured with Dulbecco's modified Eagle's medium containing various amounts of fetal bovine serum (FBS) and/or drainage fluids (DF-E or DF-L) for 1 week and cell numbers were counted. Each cell number was expressed as a ratio to that of the control culture, which was grown in media that contained 5% FBS and lacked drainage fluid. Values represent means ± SE. *$p < 0.05$ (bracketed blue lines), **$p < 0.01$ (bracketed red lines).

in fibroblasts and EC.[41] Fibroblasts appear to be the cellular source for the majority of MMP-1,[42] while neutrophils seem to provide most of the MMP-8.[43] In subcutaneous DFs, MMP-1 gradually increased up to day 6, while MMP-8 peaked on days 2–3. At all the time points examined, the levels of MMP-8 were statistically significantly higher than both MMP-1 (50–200-fold) and MMP-13 (1,000–10,000-fold). The sequential changes that we observed in MMP-1 and MMP-8 were similar to previously reported data from a study of cutaneous wound fluids.[44] Taken together, these data suggest that MMP-8 functions as the primary debriding collagenase during the acute phase of wound healing.

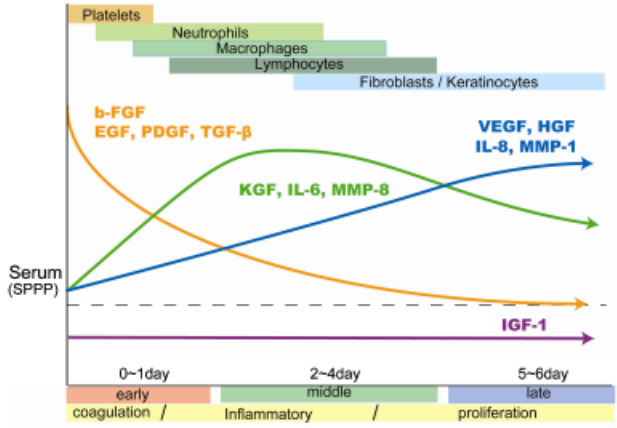

Figure 8. Summary of sequential changes in soluble factors associated with wound healing in drainage fluids from subcutaneous wounds. There are three types of sequential changes in the abundance of soluble factors that function in wound healing. First, the levels of basic fibroblast growth factor (b-FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor-β (TGF-β) are initially high and then gradually decrease. EGF and PDGF in drainage fluids in the early phase (coagulation phase) of wound healing are derived from platelets, although TGF-β is derived from various sources, and b-FGF is mainly derived from injured tissue or from cells infiltrating into wounds at the early stages. Second, keratinocyte growth factor (KGF), interleukin-6 (IL-6), and matrix metalloproteinase-8 (MMP-8) peak around days 2–4 (during the inflammatory phase). KGF is released from T lymphocytes and fibroblasts, while IL-6 seems to be discharged from the various cells involved in each phase. Third, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), IL-8, and MMP-1 are low in the early phase and gradually increase up to the late phase (proliferation phase). These factors are derived from cells involved in the later phases of wound healing, including fibroblasts and keratinocytes. Insulin-like growth factor-1 (IGF-1) is present at relatively consistent levels throughout the entire wound-healing process. MMP-13 is detected only in minimal quantities.

Potential use of wound fluids and SPRP in culture media

Because our data showed that DFs contained various growth factors that were not found in SPPP or SPRP, we tested DFs as a substitute or supplement for serum in the culturing of ASCs and dermal fibroblasts. The experiment using ASCs showed that DF-E is superior to FBS as a 5% additive of the medium containing 5% FBS, while that using dermal fibroblasts suggested that DFs may be used in a manner similar to serum. Thus, we suggest that DFs may be used as a supplement or substitute for serum in culture media, and may be able to support the growth of cell types other than the two lines examined in this study.

Recent developments in the clinical use of cultured cells (such as stem cell therapy or gene delivery) have necessitated safer preparation and manipulation of cells, which partly entails avoiding the use of animal-derived serum, tissues, and extracts. In this respect, autologous serum, cytokines, or other soluble factors could be extremely valuable. For example, ASCs isolated from liposuction aspirates of a patient could be cultured using the patient's own SPRP taken from blood and/or using DFs taken from the subcutaneous wound after liposuction. Subcutaneous wound fluids have some advantages compared with cutaneous and intraperitoneal wound fluids: cutaneous wound fluids are difficult to collect aseptically and in a large volume, and intraperitoneal ones can be obtained only through major abdominal surgery and are not aseptic in most cases.

The present results could be used as a guide in choosing the appropriate fluid supplement for cell culture, based on the specific needs of a given cell line (Table S1). Both SPRP and DFs are economical ready-made mixtures of serum (plasma) and soluble factors such as cytokines, and can also be used as raw materials for the extraction of individual soluble factor proteins. Further investigation will be necessary to provide optimized protocols for the usage of DFs and SPRP in cell culture and in factor isolation.

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Supplementary Material

The following supplementary material for this article is available for this article:

Table S1. Sources of autologous soluble factors associated with wound healing: comparison of drainage fluids, SPPP and SPRP.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1524-475X.2007.00259.x (This link will take you to the article abstract).

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