Chemokine Profile of Human Serum from Whole Blood: Migratory Effects of CXCL-10 and CXCL-11 on Human Mesenchymal Stem Cells

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Autologous human serum is used in cartilage repair and may exert its effect by the recruitment of mesenchymal stem and progenitor cells (MSC). Aim of our study was to analyze the chemokine profile of human serum and to verify chemotactic activity of selected chemokines on MSC. Human MSC were isolated from iliac crest bone marrow aspirates. Chemotactic activity of human serum made from whole blood and plasma grade serum was tested in 96-well chemotaxis assays and chemokine levels were analyzed using human chemokine antibody membrane arrays. The chemotactic potential of selected chemokines on MSC was tested dose dependently using chemotaxis assays. Human serum derived from whole blood significantly attracted human MSC, while plasma grade serum did not recruit MSC. Human chemokine antibody array analysis showed that the level of chemokines CXCL-3, 5, 7-8, 10-12, 16; CCL-2, 5, 11, 13, 16-20, 24-25, 27; as well as XCL-1 was elevated (fold change >1.5) in serum derived from whole blood compared to nonrecruiting plasma grade serum. Chemotaxis assays showed that the chemokines IP-10/CXCL-10 and I-TAC/CXCL-11 significantly recruit human MSC. PARC/CCL-18, HCC-4/CCL-16, CTACK/CCL-27, and Lymphotactin/XCL-1 showed no chemotactic effect on MSC. Therefore, human serum derived from whole blood contains chemokines that may contribute to serum-mediated recruitment of human mesenchymal progenitors from bone marrow.

Keywords: Mesenchymal Stem Cells; Serum; Chemotaxis; Chemokine; Cartilage Repair; Microfracture
INTRODUCTION

Injuries of the articular cartilage occur frequently and show no or only limited spontaneous healing or self-regeneration [1, 2]. The regenerative potential of articular cartilage is limited because potentially there is no vascular supply and no access for stem cells. A variety of surgical techniques were developed, and aimed at repair of the articular cartilage. These techniques comprise lavage, shaving, debridement, microfracture, and cell-based tissue engineering approaches [3]. The minimal invasive method of microfracturing contributes to the healing of damaged articular cartilage by opening access to the subchondral bone marrow. This access allows the influx of blood, blood-derived cells, and bone marrow-derived mesenchymal stem and progenitor cells (MSC) into the damaged cartilage region [4, 5]. This forms a cartilaginous repair tissue.

MSC are often suggested for tissue repair strategies [6], not only for cartilage repair but also for other tissues along the mesodermal lineage like bone, muscle, marrow stroma, tendon, and fat [7-10]. The advantage of using MSC in tissue repair is their high proliferation rate and the multipotent differentiation capacity. Bone marrow-derived MSC are suggested to remain undifferentiated when expanded. But with increasing cell passages, they gradually lose their proliferation and differentiation potential [11]. The population of plastic-adherent marrow cells is often not uniform and in vivo proof of typical stem cell activities like self-renewal and multipotent differentiation capacity is often scarcely to show. However, these plastic-adherent cells show homogenous populations regarding typical cell surface markers and do have multipotent differentiation capacity in vitro. Therefore, the accurate term for these cells is multipotent mesenchymal stromal cells [12].

The use of in vitro cultured mesenchymal progenitors in regenerative medicine has been reported in several animal and clinical studies, e.g., in restoration of articular cartilage surfaces in osteoarthritic lesions [13], for cartilage repair with bone marrow mesenchymal cells embedded in collagen [14, 15], regeneration of mandibular bone [16, 17], for spinal fusion surgery [18], and for cardiovascular diseases [19]. Biopsies and extensive manipulation of MSC are required for stem cell-based therapies. Therefore, these therapies may be stressful for the patient as well as time and labor intensive.

Newer, cell-free one-step approaches for cartilage repair favor microfracture treatment and subsequent covering with bioactive scaffolds that attract stem cells into the scaffold, i.e., by serum or chemotactants, and guide the progenitors to form cartilage repair tissue [20, 21]. In particular, the migratory potential of human serum may be of special interest in cartilage or tissue repair. Fetal bovine serum is known for its dose-dependent chemotactic potential on MSC and chondrocytes [22].

MSC have been reported to be recruited by fibroblast growth factors, bone morphogenetic proteins, platelet-derived growth factors, vascular endothelial growth factors, and insulin-like growth factors [22-25], as well as chemokines [26]. However, it has still to be elucidated which particular factor or factors may contribute to the recruitment of cells by blood-derived serum. Components of serum that are suggested to play an important role for cell and MSC migration are a variety of growth and differentiation factors and chemokines.

Chemokines are chemotactic cytokines and the nomenclature of nearly 50 known chemokines is based on the presentation of invariant cysteine residues within the mature protein and divides chemokines and their G-protein-coupled chemokine receptors into CC, CXC, XC, and CX3C subfamilies [27-29]. In recent years, the field of regenerative medicine and tissue engineering focused on stem cell cultivation and characterization as well as stem cell migration. This approach has lead to a variety of chemokines being reported to stimulate the migration of human mesenchymal stem and progenitor cells [21, 30-34].

Here we show that human serum derived from whole blood recruits human bone marrow-derived stem cells and contains a variety of chemokines. Sera derived from whole blood show elevated levels of particular chemokines, compared to nonrecruiting plasma grade serum. The selected chemokines, CXCL-10 and CXCL-11, recruited human MSC in chemotaxis assays, while XCL-1, CCL-16, CCL-18, and CCL-27 had no chemotactic effect on MSC. These results suggest that chemokines contribute to the serum-mediated migration of mesenchymal progenitor cells in tissue repair.

MATERIALS AND METHODS

Isolation and Culture of Human MSC

Human adult MSC were isolated from iliac crest bone marrow aspirates of healthy donors (n = 15; 7 females, 8 males; age: 38-86; mean age: 59.5) as described previously [35]. Bone marrow samples were derived from individual donors examined to exclude hematopoietic neoplasmas and were histologically diagnosed as normal. The study was approved by the ethical committee of the Charité Universitätsmedizin Berlin. In brief, MSC from aspirates (4 ml per sample) were purified using a percoll gradient of a density of 1.073 g/ml and suspended in Dulbecco's modified Eagle (DME)-medium (Biochrom) containing 2 ng/ml basic fibroblast growth factor (bFGF, Tebu) and 10% fetal bovine serum (FBS, Hyclone). Cells were plated at a density of 300,000 cells/cm², medium was exchanged after 72 hr and then every 2 to 3 days thereafter. Reaching 90% confluence, cells were detached by the addition of phosphate buffered saline (PBS, Biochrom) containing 0.05% trypsin-EDTA (Biochrom) and replated at a density of 4,000 cells/cm².

Fluorescent-Activated Cell Sorting (FACS) Analysis

Typical MSC-related cell surface antigens were analyzed as described previously [21, 31]. In brief, single cell suspensions (passage 3) were washed twice in PBS supplemented with bovine serum albumin (BSA, 0.5% v/v) and were incubated.
with primary staining reagents for 10 min on ice (10^6 cells/ml in PBS/0.5% BSA). Presence of CD14, CD34, CD73, and CD166 was analyzed with R-Phycocerythrin labeled mouse antihuman antibodies (all Pharmingen), while mouse antihuman antibodies raised against CD45 and CD105 (provided by the German Rheumatism Center) were labeled with fluorescein isothiocyanate. Labeled MSC were washed in PBS/0.5% BSA and analyzed using a FACSCalibur cytometer (Becton Dickinson). Dead cells and debris were excluded by forward and side scatter. For analysis, CellQuest software (Becton Dickinson) was used.

Analysis of Serum-Mediated Chemotaxis

Chemotaxis of MSC (pool of cells derived from 7 donors; 2 females, 5 males; age: 49–70; mean age: 60.8) upon stimulation with human serum (HS) made from whole blood (n = 5, German Red Cross) and pharma grade human serum (Bioseko, Biotest) were measured in 96-multiwell format ChemoTX® plates (Neuroprobe) with 8 μm polycarbonate membranes as described previously [21, 36]. Briefly, 3 x 10^4 MSC (passage 3) were resuspended in DME-medium and seeded in the upper wells. The lower wells were supplied with DME-medium containing 10% HS from whole blood or 10% pharma grade human serum. As control, DME-medium without serum was used. The assay was performed in triplicates. The chamber was incubated for 20 hr at 37°C. After removal of nonresponding MSC on top of the filter, cells that migrated through the membrane were fixed in methanol/acetone, stained with Hemacolor (Merek), and enumerated microscopically by counting the number of stained cells in three representative fields. Statistical analysis was carried out utilizing one-way analysis of variance (ANOVA) and all pairwise multiple comparison procedures according to Duncan’s method. Significance was considered at a p value of p < 0.05.

Human Chemokine Antibody Membrane Array Analysis

According to the manufacturer’s instructions the level of 38 chemokines was determined in HS from whole blood (n = 5) and pharma grade serum using human chemokine antibody membrane arrays (RayBiotech), according to the manufacturer’s recommendations. Protein content was measured using the BCA (bicinchoninic acid) protein assay (Sigma). After blocking, membranes were incubated for 2 hr with 6 mg/ml HS derived from whole blood or pharma grade human serum diluted in blocking buffer (6 mg/ml serum corresponds to cell culture medium with 5–10% serum) at 20°C. Detection of chemokines was performed according to the manufacturer’s recommendations by biotin-conjugated antibodies raised against the particular chemokines and horseradish peroxidase-conjugated streptavidin. Chemokines were detected by chemiluminescence and the membranes were briefly exposed to X-ray films (Amersham) for 2.5 min.

Images were digitized at a resolution of 300 dpi and spot intensity was determined densitometrically by using Photoshop as described previously [37]. In brief, a color was defined representing the background color of the array in a given area (negative control). For each spot, the number of stained and unstained pixel were determined within a given area. For normalization of arrays, signal values of chemokines were divided by the mean value of the positive control. The fold change (FC) was determined by dividing the signal value of the chemokine in serum made from whole blood by the signal value of the respective chemokine found in pharma grade serum. Chemokine levels were considered to be different, when the respective chemokine level showed a FC > 1.5 or FC < -1.5 in each individual serum derived from whole blood compared to pharma grade serum.

Analysis of Chemokine-Mediated Chemotaxis

Chemotactic activity of CXCL-10 (IP-10, PeproTech, n = 9), CXCL-11 (I-TAC, PeproTech, n = 9), CXCL-1 (Lymphotactin, PeproTech, n = 9), CCL-27 (CTACK, PeproTech, n = 9), CCL-16 (HCC-4, PeproTech, n = 6), and CCL-18 (PARC, PeproTech, n = 6) was measured in 96-well chemotaxis assay with 8 μm polycarbonate membranes. Briefly, 3 x 10^4 MSC (passage 3) were resuspended in DME-medium containing 0.1% FBS and seeded in the upper wells. The lower wells were supplied with 0 nM to 1,000 nM of the respective chemokine in DME-medium containing 0.1% FBS. Chemokinesis was excluded by testing cell migration with 50 nM or 1,000 nM chemokine in DME-medium containing 0.1% FBS added to both the upper and lower wells in triplicate. After performing the assay and enumeration of migrated cells (see Analysis of Serum-Mediated Chemotaxis), data were normalized by calculating the “migration index” (ratio between migrated MSC on chemokine treatment and migrated MSC in the absence of the chemokine) and ANOVA was carried out.

The one-way procedure was applied when a normal distribution of values was given and the Kruskal-Wallis ANOVA method when not. Significant differences between tested chemokine doses and control were determined by Dunn’s method. For isolating the groups that differ significantly from the others, the all-pairwise multiple comparison procedure was performed according to Student-Newman-Keuls. Differences were considered significant at p < 0.05.

Analysis of Chemokine Receptor CXCR3

For analysis of CXCR3 expression in MSC, three independent MSC preparations were used for gene expression and immunohistochemical analysis [31]. In brief, total RNA (3 μg) of MSC (n = 3, passage 3) were reversely transcribed with the iScript cDNA synthesis kit according to the manufacturer’s recommendations (Bio-Rad). The relative expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs99999905_m1, Applied Biosystems) was used to normalize marker gene expression. The expression levels of CXCR3 (forward: CCC GCA ACT GGT GCC GAG AAA G; reverse:
AGG CGC AAG AGC AGC ATC CAC AT; product size: 148 base pairs) were determined in triplicates (n = 3) using the SYBR Green PCR Core Kit (Applied Biosystems) and are given as the percentage of the housekeeping gene GAPDH.

For immunohistochemistry, MSC (passage 3) were cultured in 8-well chamber slides (Lab-Tek) for 24 hr. Cells were fixed with ice-cold methanol/acetone (1:1 v/v) and incubated with mouse-antihuman CXCR3 antibodies (R&D Systems) for 30 min. Mouse IgG served as control. Subsequently, horseradish-labeled secondary antibodies were used for detection, followed by counterstaining with hematoxylin.

RESULTS

MSC Migration

Human bone marrow-derived mesenchymal stem cells were analyzed routinely for the presence of typical MSC-related cell surface antigens by flow cytometric analysis. Cells showed the typical antigen profile of MSC and were positive for CD73, CD105, and CD166. MSC were negative for the antigens CD14, CD34, and CD45 (Figure 1).

The migration potential of MSC on stimulation with HS derived from whole blood and pharma grade HS was analyzed in transwell chemotaxis assays (Figure 2). Using HS from whole blood as a chemoattractant between 2,810 and 8,831 MSC were recruited serum-dependently. In contrast, only up to 48 MSC migrated when pharma grade serum was used as a chemoattractant. In controls, between 401 and 819 MSC migrated spontaneously in the absence of serum. Compared to controls, the recruitment of MSC mediated by serum from whole blood was significantly (p < 0.05) enhanced.

FIG. 1. Flow cytometric analysis of adult human bone marrow-derived mesenchymal stem cells (MSC). Flow cytometric analysis demonstrates that culture expanded MSC (passage 3) are positive for the antigens CD73, CD105, and CD166, while cells are negative for CD14, CD34, and CD45.

FIG. 2. Chemotactic response of human MSC upon stimulation with human serum derived from whole blood. Independent human sera (serum 1–5) derived from whole blood significantly (p < 0.05) recruited human MSC derived from bone marrow. The bars show the mean and SD.
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Chemokine profile of humanserum. Chemokine antibody array membranes show the chemokine profile of human serum derived from whole blood (A) and human pharma grade serum (B). The configuration of the array membrane is given in (C).

Different Chemokine Levels in Serum
To quantify chemokine levels in HS from whole blood and pharma grade serum, chemokine antibody arrays were used (Figure 3). In general, array membranes treated with serum from whole blood (Figure 3A) showed more intensive spots compared to pharma grade serum (Figure 3B). The array configuration representing 38 chemokines in duplicates is given in Figure 3C. In all human sera made from whole blood, the chemokines CXCL-3, 5, 7–8, 10–12, 16; CCL-2, 5, 11, 13, 16–20, 24, 25, and 27 and XCL-1 were elevated with a fold change (FC) >1.5 compared to pharma grade serum (Table 1). The chemokines CX3CL-1, CXCL-1, 6, 9, 13 and CCL-1, 3, 4, 7–8, 15, 22–23, 26, and 28 were not elevated in HS and showed a FC <1.5, at least in one serum sample derived from whole blood compared to pharma grade serum. Interestingly, no chemokine was robustly reduced in serum derived from whole blood compared to pharma grade serum. Most elevated chemokines were CCL-25, CXCL-16, CCL-19, and CCL-17 with a FC of 27 up to 678. Since these chemokines are known to recruit MSC (e.g., CCL-25; [34, 38]), chemokines were selected for further chemotactic analysis that showed a low fold change and represent the CXC, CC, and XC subgroups.

Chemokine-Mediated Chemotaxis
The migratory effect of the chemokines CXCL-10, CXCL-11, XCL-1, CCL-16, CCL-18, and CCL-27 on human MSC derived from iliac crest bone marrow was determined in chemotaxis assays (Figures 4 and 5). CCL-16 showed a mean migration index of 0.47 to 1.02 (Figure 4A). In controls, between 498 and 1477 MSC migrated spontaneously, while 1130 MSC migrated upon treatment with 1 nM CCL-16. Fully 830 MSC migrated after stimulation with 1,000 nM of the chemokine (data not shown). Treatment of MSC with 250 nM CCL-16 significantly (p = 0.031) reduced the migration of MSC compared to nonstimulated controls with a mean migration index of 0.47. CCL-27 (Figure 4B) did not significantly enhance migration of MSC. But 1,000 nM CCL-27 stimulated the highest number of MSC with a migration index of 1.3 and up to 1,029 migrating MSC. Treatment of MSC with 100 nM CCL-27 significantly (p < 0.001) reduced the migration of MSC compared to nonstimulated controls and compared to MSC treated with 1 to 50 nM and 250 to 1,000 nM of the chemokine. The migration index of MSC treated with 100 nM CCL-27 was 0.31 representing 821 migrating MSC in controls and 275 after stimulation with the chemokine. CCL-18/PARC (Figure 4C) and

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### TABLE 1
Chemokine profile and fold changes of serum derived from whole blood

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**FIG. 4.** Stimulation of human MSC with CCL-16, CCL-18, CCL-27, and XCL-1. The chemotactic response of chemokines is given by the migration index. CCL-16 (A) did not recruit MSC and significantly (*, p < 0.05) reduced MSC migration at 250 nM compared to controls. Stimulation of MSC with CCL-27 did not enhance cell migration (B). MSC migration was significantly reduced by 100 nM CTACK/CCL-27 compared to negative control (*, p < 0.05) and to all other tested doses (#, p < 0.05). The chemokines CCL-18 (C) and XCL-1 (D) showed no chemotactic effect on MSC. The bars show the mean and SD.
lymphotactin/XCL-1 (Figure 4D) did not recruit MSC (CCL-18, p = 0.463; XCL-1 p = 0.607). The chemokines CCL-16, CCL-18, CCL-27, and XCL-1 did not recruit MSC.

High doses of the chemokines CXCL-10/IP-10 and CXCL-11/I-TAC recruited human MSC (Figure 5). CXCL-10 showed a migration index of 3.41 when 1,000 nM of the chemokine was used for stimulation (Figure 5A). Fully 1,000 nM CXCL-10 significantly (p < 0.05) recruited MSC compared to nonstimulated controls and to MSC treated with 1 to 750 nM of the chemokine. Nonstimulated controls showed spontaneous migration of 979 to 3,789 MSC, while 1,000 nM CXCL-10 recruited up to 13,488 MSC (Figure 5B). High doses of 1,000 nM CXCL-11 showed a migration index of 3.37 and significantly (p < 0.05) recruited MSC compared to nonstimulated controls and to MSC treated with 1 to 750 nM of the chemokine (Figure 5C). In the absence of CXCL-11, 32 to 1,317 MSC migrated, showing a less spontaneous migration potential than MSC derived from independent donors used for the CXCL-10 chemotaxis assay. A total of 1,000 nM CXCL-11 recruited up to 3,789 MSC (Figure 5D).

Human MSC showed low levels of CXCR3 that is the receptor for CXCL10 and CXCL11 (Figure 6). As assessed by PCR analysis, independent MSC showed low expression levels of CXCR3 (mean, 0.0019% to 0.0024% of the expression level of GAPDH) (Figure 6A). Immunohistochemical staining showed cellular presence of CXCR3 (Figure 6B).
DISCUSSION

Here we show that human sera derived from whole blood contain elevated levels of distinct chemokines compared to human grade human serum. Human serum derived from whole blood robustly recruits human MSC derived from bone marrow. The chemokines CCL-16, CCL-18, CCL-27, and XCL-1 did not recruit MSC, while CXCL-10 and CXCL-11 that were elevated in sera from whole blood effectively recruited human MSC. This suggests that serum derived from whole blood contains functionally active chemokines that may contribute to the migratory effect of serum on human MSC.

MSC are a well known cell type in regenerative medicine [10, 39, 40] with the capacity to develop into a variety of mesenchymal tissue like cartilage and bone [7, 8]. Stimulating the migration of mesenchymal stem and progenitor cells with serum [21, 22, 41] may open new opportunities in cartilage repair. The formation of cartilage repair tissue mediated by the microfracture technique depends on damage of the subchondral bone [4]. After injury of the bone, influx of blood, blood-derived cells and bone marrow-derived progenitor cells as well as on potential growth factors and chemokines present in the blood and in the bone marrow occurs [42, 43]. MSC may be flushed into the defect by blood [5] or may be actively recruited by, for instance, synovial fluid [36].

Recently, we have shown that a cell-free implant composed of a resorbable polyglycolic acid felt and hyaluronan immersed in autologous serum may recruit MSC to the cartilage defect area and improves cartilage healing compared to the standard microfracture technique [20]. It has been suggested that autologous serum in combination with partial autologous fibrin glue improves the recruitment of MSC after microfracture [44]. Recent studies showed that mesenchymal progenitor cells harvested from bone marrow can be attracted by the serum containing blood clot in combination with a chitosan scaffold [45, 46]. Interestingly, as shown here, plasma grade serum recruited less MSC and showed lower levels of particular chemokines than HS derived from whole blood. The presence of less chemokines may contribute to the reduced migratory effect of plasma grade serum on MSC. For safety reasons, plasma grade serum is virus inactivated by heating, UV-radiation, and addition of beta-propiolacton. UV-irradiation may lead to inactivation of distinct chemokines and growth factors that mediate cell recruitment. In addition, beta-propiolacton is toxic and may affect MSC, thus leading to a reduced MSC migration potential. However, macroscopically, apoptotic cells or morphological aberrations were not observed in MSC stimulated with plasma grade serum. Obviously, HS from whole blood effectively recruited MSC, in contrast to plasma grade serum. Therefore, autologous serum may improve cell recruitment, contribute to tissue repair, and may be a key element of scaffold-based tissue regeneration approaches that are suggested for clinical improvement of the microfracture technique [20, 47].

Since the composition of HS remains unclear, it still has to be elucidated which particular factor or factors mediate the serum-dependent recruitment of MSC. As shown here, human sera derived from whole blood show elevated levels of the chemokines CXCL-3, 5, 7-8, 10-12, 16; and CCL-2, 5, 11, 13, 16-20, 24-25, 27 as well as XCL-1 that may be, at least in part, key mediators in serum induced chemotaxis of bone marrow derived mesenchymal stem and progenitor cells. In recent studies, it is a variety of these CC and CXC chemokines like CCL-5, CCL-19, CCL-25, CXCL-7, CXCL-12, and CXCL-16 recruit human mesenchymal progenitors derived from bone marrow [21, 26, 31, 34, 38].

Other chemokines, as shown here, do not recruit MSC and may potentially inhibit the migration of MSC. However, the chemokines CXCL-10/IP-10 and CXCL-11/T-AIC effectively recruit MSC. The migratory effect of CXCL-10 is in concordance with a recent report that showed that the proinflammatory cytokines interferon γ (IFNy) and tumor necrosis factor α (TNFα) stimulate the synthesis of CXCL-10 and enhance the CXCL-10-mediated migration of MSC [48]. In the same report, MSC migration was induced by CXCL12 and CXCL1, while co-stimulation with TNFα/IFNy as well as IFNy alone enhanced the CXCL12-mediated migration and reduced the CXCL1-mediated recruitment of MSC. The doses of CXCL-10 and CXCL-11 that stimulated MSC migration were high and not physiological as known from our own studies that showed that nonphysiological doses of CXCL-8, CXCL-12, CXCL-13, CCL-2, and CCL-25 are needed for effective recruitment of human mesenchymal progenitors derived from bone marrow and periosseum [21, 41]. In these reports, progenitors marginally expressed a variety of chemokine receptors including the CXCL-10/CXCL-11 receptor CXCR3, as also shown in the present study. This may explain the high doses of chemokines needed for in vitro recruitment of MSC.

In vivo, it is most likely that low and physiological doses of chemokines are needed for cell recruitment and tissue repair as shown recently for SDF-1alpha/CXCL-12. Using a mouse myocardial infarct model, CXCL-12 embedded in a patch composed of fibrin and polyethylene glycol recruited murine stem cells into the infarct area and improved ventricular function in mice with postinfarction ventricular remodeling [49]. Thinking of the clinical application of chemokines for cartilage repair, the most effective doses for in situ recruitment and repair tissue formation have to be elucidated. In addition to that, it has to be taken into account that a given chemokine may have unwanted effects on tissue formation. For instance, the chemokines RANTES/CCL-5, MIP-1alpha/CCL-3, and MIP-1beta/CCL-4 are suggested to play a key role in cartilage degradation [50].

Therefore, their use may be problematic in cartilage regeneration approaches. Further studies have to elucidate in detail which chemotactically active chemokines or other factors are involved in serum-mediated MSC recruitment and which chemokines...
influence or benefit tissue repair in vivo. In particular, studies using serum derived from patients with cartilage injuries are needed that may underline the role of distinct chemokines like CXCL-10 and CXCL-11 in MSC recruitment in cartilage repair.

CONCLUSION
In the present study we showed that human serum made from whole blood recruits bone marrow-derived mesenchymal stem cells, while pharma grade serum does not. Serum from whole blood revealed elevated levels of particular chemokines compared to pharma grade serum. The chemokines CXCL-10 and CXCL-11 effectively stimulated the migration of MSC. These results suggest that serum contains chemokines that may contribute to tissue repair by recruiting human mesenchymal progenitors from bone marrow.

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Declaration of Interest
GIK, ME, KN, and CK are employees of TransTissue Technologies GmbH (TTT). MS is a consultant of TTT. TTT develops products in the field of regenerative medicine for mesenchymal tissues. All other authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of this article.

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