In Vitro Cell Motility as a Potential Mesenchymal Stem Cell Marker for Multipotency

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ABSTRACT

Mesenchymal stem cells (MSCs) are expected to have a fundamental role in future cell-based therapies because of their high proliferative ability, multilineage potential, and immunomodulatory properties. Autologous transplantsations have the “elephant in the room” problem of wide donor variability, reflected by variability in MSC quality and characteristics, leading to uncertain outcomes in the use of these cells. We propose life imaging as a tool to characterize populations of human MSCs. Bone marrow MSCs from various donors and in vitro passages were evaluated for their in vitro motility, and the distances were correlated with the adipogenic, chondrogenic, and osteogenic differentiation potentials and the levels of senescence and cell size. Using life-image measuring of track lengths of 70 cells per population for a period of 24 hours, we observed that slow-moving cells had the higher proportion of senescent cells compared with fast ones. Larger cells moved less than smaller ones, and spindle-shaped cells had an average speed. Both fast cells and slow cells were characterized by a low differentiation potential, and average-moving cells were more effective in undergoing all three lineage differentiations. Furthermore, heterogeneity in single cell motility within a population correlated with the average-moving cells, and fast- and slow-moving cells tended toward homogeneity (i.e., a monotonous moving pattern). In conclusion, in vitro cell motility might be a useful tool to quickly characterize and distinguish the MSC population’s differentiation potential before additional use.

INTRODUCTION

In recent years, tissue engineering and cell-based therapies have developed into fast expanding disciplines, and mesenchymal stem cells (MSCs)—among all cell types potentially available for such therapies—have emerged as the favorite candidates. These adult stem cells are characterized by multiple capacities, including fast clonal expansion [1, 2], secretion of trophic and immunomodulatory factors [3], and differentiation into several lineages [4]. Although MSCs have been isolated from diverse adult tissues, many future therapeutic applications will be based on MSCs isolated from bone marrow stroma owing to its accessibility [5]. Currently, the registry and results database Clinicaltrials.gov lists more than 400 ongoing clinical trials for MSC therapies in humans. The treatment targets are very broad, including diseases and conditions of different origins, from orthopedic and neurological, to cardiac and immune-mediated. However, a common unknown—the efficacy of these trials—depends strongly on the intrinsic composition of cell preparations. MSC cultures are characterized by a heterogeneous mixture of cells at different stages of commitment and potential differentiation [6]. Zhang et al. showed that administration of single clonally purified MSCs were more efficient in the repair of infarcted myocardium compared with the original MSC preparation from where the clone was derived [7].

Despite several recent reports [8–10], our knowledge about the underlying variability in the intrinsic differentiation capacity and the hierarchical cell population relationships within MSC preparations is currently still limited. The manifestation of such heterogeneity is the cell morphology of MSC preparation, ranging from spindle shaped to small and regular shapes [11]. The first reports on MSC hierarchy described an initial loss of adipogenic, followed by chondrogenic and, finally, osteogenic potential of cell progenitors [12]. The morphology of the cells alone, however, seems to be challenged by the changing cell density and proliferation to offer reliable prediction of differentiation potential [13]. Recent studies have also showed that MSC heterogeneity is more complex and all combinations of tripotent, bipotent, and unipotent progenitors are present at the same time [14]. However, subsequent work showed that only two combinations of lineage commitment are detectable by clonal isolation—osteogenic/adipogenic progenitors and osteogenic/chondrogenic progenitors, and the unipotent progenitors had only osteogenic
potential [15]. Obviously, the development of a standardized protocol for characterizing MSCs is important to evaluate the odds of a successful cell therapy.

We hypothesized that in vitro cell motility could be used to develop a fast method to characterize MSC populations regarding their clinical potential. Cell movement has been largely studied in wound healing [16], cell signaling [17], immunology, and cancer research [18]. In vitro, cell movements depend on the cell type, confluence, and chemical or mechanical stimulation, and random crawling motion (motility) must be distinguished by directional movements (migration). Different cell types can be characterized by different speed motilities. Thus, human fibroblasts move slower (12–60 μm/h) than neutrophils, which are the fastest-moving leukocytes (900–1,200 μm/h) [19].

In the present study, we investigated by time-lapse microscopy the in vitro motility of human MSCs isolated from different donors at various culture passages to determine a correlation between cell movement and differentiation into adipogenic, chondrogenic, and osteogenic lineages, assessed by quantification of gene and protein expression. Cell motility was recorded for 24 hours, and the positions of individual cells were then marked on consecutive images. In parallel, we also compared the cell motility with cell size and cell senescence—the latter marked by increased expression of senescence-associated β-galactosidase [20].

**MATERIALS AND METHODS**

**MSC Isolation and Culture**

Fresh bone marrow (BM) samples were obtained from the iliac crest of the donors during surgery after they had provided informed consent. Harvest of the BM sample had been previously approved by the ethics committee of canton Lucerne. MSCs were isolated from the BM of 23 donors (minimum age 17 years; maximum age 67 years; average age 46 ± 15 years). The BM aspirates were diluted in 3.8% sodium citrate and phosphate-buffered saline (PBS) and then filtered through a 100-μm cell strainer to remove any clots (Falcon; BD Biosciences, San Diego, CA, http://www.bdbiosciences.com). Mononuclear cells were separated by Ficoll gradient centrifugation (density 1.077 g/ml; GE Healthcare, Little Chalfont, U.K., http://www.gehealthcare.com) in a Leucosep tube (Greiner Bio-One, Frickenhausen, Germany, http://www.gbo.com/en) at 800g for 15 minutes, washed with PBS, centrifuged again at 210g for 10 minutes, resuspended in PBS, and counted using trypan blue dye in a single-use Neubauer chamber (C-Chip Typ Neubauer; Carl Zeiss, Jena, Germany, http://www.zeiss.com). The cells were plated in tissue culture flasks in α-minimum essential medium (Bioconcept, Allschwil, Switzerland, http://www.bioconcept.ch), supplemented with 10% fetal bovine serum (FBS) (Bioconcept), 100 units/ml penicillin with 100 mg/ml streptomycin and 2.5 μg/ml amphotericin B (both Gibco, Grand Island, NY, http://www.invitrogen.com) at 37°C in a humid atmosphere containing 5% CO2. After 2 days, nonadherent cells were discarded, and adherent cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM)/Ham’s F12 (Bioconcept) supplemented with 10% FBS, 100 units/ml penicillin with 100 mg/ml streptomycin, 2.5 μg/ml amphotericin B, and 5 ng/ml recombinant basic fibroblast growth factor (Peprotech, Rocky Hill, NJ, http://www.peprotech.com) with the medium changed 3 times weekly.

**Flow Cytometry With MSC Markers**

MSCs were sampled at 1 × 10^6 cells per tube to investigate the proportion of CD44-, CD90-, and CD105-positive and CD14-negative cells. The cells were incubated with CD14-fluorescein isothiocyanate (FITC) (NB100-77759; Novus Biologicals, Littleton, CO, http://www.novusbio.com), CD44-FITC (NBPI-41278; Novus Biologicals), CD90-FITC (NBPI-96125; Novus Biologicals), and CD105-FITC (MACA1557A488T; AbD Serotec, Raleigh, NC, http://www.ab-direct.com) antibodies in PBS plus 1% FBS for 1 hour at 20°C, washed, and resuspended in PBS. Cell fluorescence was evaluated by flow cytometry in a Cell Laboratory QuantaSC instrument (Beckman Coulter, Fullerton, CA, http://www.beckmancoulter.com), and the data were analyzed using Cell Laboratory QuantaSC MPL analysis software, version 1.0 (Beckman Coulter).

**In Vitro Cell Motility Tracking and Cell Area Measurement of MSCs**

MSC populations at various in vitro passages (from passage [P]3 to P11) were plated at a density of 5.6 × 10^3 cells per cm^2. After 3 hours, the movements of the adherent cells were recorded using phase-contrast microscopy and an inverted microscope equipped with a high-sensitive camera (Olympus, Tokyo, Japan, http://www.olympus-global.com) at 40× magnification. The interval between each acquisition was 10 minutes, and images were acquired using the excellence software program (Olympus) during a 24-hour period. The same program was used to manually measure the cell area of the individual MSCs.

Video sequences were analyzed using ImageJ (NIH, Bethesda, MD, http://www.nih.gov/ij) and the plugin MTrackJ, which allows manual tracking of individual cell trails. Analyses were only made for cells moving within the plane focus. The full length of the track was determined as the distance from the first point to the last point of the track, and the cell speed was measured as mm/day.

**Senescence-Associated β-Galactosidase Assay**

Immediately after tracking, the cells were fixed with 2% formaldehyde and 0.2% glutaraldehyde (both AppliChem, Omaha, NE, http://www.applichem.com) in PBS and incubated overnight at 37°C in a freshly prepared staining solution consisting of 150 mM sodium chloride, 2 mM magnesium chloride, 30 mM citric acid/phosphate buffer, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide (all AppliChem), and 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) at pH 6.0 [21]. After washing with PBS, the cells were counterstained with hematoxylin (LuBioScience GmbH, Lucerne, Switzerland, http://www.lubio.ch), and senescence-associated β-galactosidase assay-positive cells were enumerated using bright field microscopy and compared with their respective measured tracks.

**MSCs In Vitro Differentiation Into Chondrogenic, Osteogenic, and Adipogenic Phenotypes**

The potential of MSCs to differentiate into chondrogenic, osteogenic and adipogenic lineages was investigated. The cultures were stimulated for 2 weeks with the appropriate differentiation media as described below.
Chondrogenic Differentiation

Cubes (3 mm per side) from a sponge-shaped medical device (Spongostan; Ferrosan Medical Devices, Soeberg, Denmark, http://www.ferrosanmedicaldevices.com) composed of gelatin were cut and used as scaffold material to support cellular growth [22]. MSCs (72,000 cells per construct) were seeded in the cubes and kept for 30 minutes to allow adhesion before the addition of medium. The MSC constructs were maintained in chondrogenic medium consisting of DMEM/Ham’s F12, 2.5% FBS, 100 units/ml penicillin, 100 mg/ml streptomycin, 2.5 μg/ml amphotericin B, 40 ng/ml dexamethasone (Sigma-Aldrich), 50 μg/ml ascorbic acid 2-phosphate (Sigma-Aldrich), 50 μg/ml l-proline (Sigma-Aldrich), 1 × insulin-transferrin-selenium X (Gibco), and 10 ng/ml transforming growth factor-β1 (PeproTech). GAG accumulation was quantified with Alcian blue binding as- say after 6 hours of digestion of 3 constructs per sample at 60°C with 125 μM papain (Sigma-Aldrich) in 5 mM L-cysteine-HCl (Fluka), 5 mM Na-citrate, 150 mM NaCl, and 5 mM EDTA (all AppliChem). GAG accumulation was determined by binding to Alcian blue (Fluka). Absorption was measured at 595 nm and quantified against the chondroitin sulfate (Sigma-Aldrich) reference standards [23].

Osteogenic Differentiation

MSCs cultured in a monolayer at a density of 5 × 10⁵ cells per cm² were differentiated using the StemPro Osteogenesis Differentiation Kit (Gibco). The von Kossa stain was used to identify mineralization deposits in the cell culture; under illumination, 5% AgNO₃ (AppliChem) was reduced to metal silver (black stain).

The calcium content was determined using the Calcium CPC LiquiColor test kit (Stanbio Laboratories, Boerne, TX, http://www.stanbio.com) as follows. The cells were washed twice with PBS and incubated with 0.5 N HCl for 30 minutes at room temperature. Next, O-cresolphthalein complex was added, and the calcium content in the liquid was measured (absorbance at 595 nm) and quantified with standards.

Adipogenic Differentiation

MSCs were cultured in monolayers at a density of 5 × 10⁵ cells per cm² under 2 different culture conditions: an adipogenesis-inducing medium (basal medium [DMEM/Ham’s F12 plus GlutaMAX, 2.5% FBS, 100 units/ml penicillin, 100 mg/ml streptomycin, 2.5 μg/ml amphotericin B] supplemented with 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 0.5 mM indomethacin, and 170 mM insulin; all Sigma-Aldrich) and adipogenesis maintenance medium (basal medium supplemented with 170 mM insulin). Lipid droplets were revealed by staining with Oil Red O (Sigma-Aldrich), and the dye content was quantified after isopropanol elution and spectrophotometry by measuring the absorbance at 520 nm.

Statistical Analysis

The cell surface sizes of the MSCs were compared between motility groups using the Wilcoxon rank sum test. The proportion of senescence cells between the motility groups was investigated using the chi-square test. The differentiation potential versus cell motility was depicted in scatterplots separately for adipogenic, chondrogenic, and osteogenic differentiation potentials.

Scatterplot smoothers with 95% confidence bands were produced via semiparametric regression models using the mixed model representation of penalized splines as implemented in the SemiPar package in R (R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). The cell motility in each population was summarized using the mean ± SD. In order to investigate the relation between these two statistical measures, the coefficient of variation was calculated (SD divided by the mean) and compared with mean motility measurements in a scatterplot. Statistical tests were computed using SPSS, version 14.0, for Windows (IBM Corp., Armonk, NY, http://www-01.ibm.com/software/analytics/spss/); scatterplots and scatterplot smoothers were computed using R, version 2.14.2, for Windows (R Foundation for Statistical Computing).

RESULTS

MSC Characterization and Differentiation

The bone marrow-isolated MSCs were characterized by flow cytometry analysis with the positive mesenchymal stem cell markers CD44 (Fig. 1A), CD90 (Fig. 1B), and CD105 (Fig. 1C) and the negative monocyte marker CD14 (Fig. 1D). In the representative sample, of the immunolabeled MSCs, 99.1% were positive for CD40, 95.3% for CD90, and 99.3% for CD105 and lacked expression of CD14 (<1% positive). Furthermore, MSCs were histologically tested for their ability to differentiate in adipogenic (Fig. 1E, top row), chondrogenic (Fig. 1E, bottom row, left), and osteogenic (Fig. 1E, bottom row, right) phenotypes, stained with the Oil Red O, Alcian blue, and von Kossa methods, respectively.

Cell Tracking

We used 2-dimensional time-lapse microscopy to record the cell movements in cultures of human MSCs isolated from 23 donors at various culture passages (ranging from P3 to P11). For each MSC population, 70 cells were tracked. The results showed a variability between samples in the median speed and the distribution pattern of the cells. Representative photographs show the tracking plot of the slowest-moving population (median speed of ~0.1 mm/day; Fig. 2A) and the fastest (median speed of ~0.9 mm/day; Fig. 2B), for which the cell tracks (each colored line represents a single cell) were markedly longer for the fast-moving cells than for the slow-moving cells.

The entire collection of cell tracks from all analyzed MSC populations (n = 1,610) followed a polynomial of degree 4 distribution (r² = 0.87), with its peak at 0.38 mm/day (Fig. 2C).

Cell Tracking Versus Cell Morphology, Cell Size, and Senescence

The motility of the MSCs correlated inversely with “fried egg” cell morphology (Fig. 3A), cell size (Fig. 3B), and cell senescence (Fig. 3C). We observed that small and round cells were the fastest (faster than 1 mm/day) compared with the cells with a larger/“fried egg” aspect, asymmetrical shape, and more marked nuclear appearance on phase-contrast microscopy (slower than 0.5 mm/day). Spindle-shaped cells, similar to fibroblasts, had an average speed within the range of 0.5–1.0 mm/day.

In accordance with these observations, the average cell size of the MSCs in the slowest group (0–0.5 mm/day) had a larger cell surface of ~6,000 μm² compared with the middle group.
(0.5–1.0 mm/day) of ∼5,000 μm² and the fastest group (1.0–1.5 mm/day) of ∼3,500 μm² (p < .05).

The divergence in cell motilities between MSCs also correlated with the amount of senescent cells present in the population, as evaluated using the senescence-associated β-galactosidase assay. In the slowest group, the percentage of senescent cells normalized to the total amount of the cells within the group was 39% (p < .05); in the middle group, it was 26% and in the fastest group, 11%.

Cell Tracking Versus Differentiation Potential

After 14 days, differentiation of the MSC populations was quantified using (a) a calcium deposition assay for osteogenesis, (b) an Alcian blue precipitation assay for chondrogenesis, and (c) Oil Red O staining measurement for adipogenesis. The collected results were directly compared with the distribution of cells (represented in the graphs as single dot) versus the number of cells with the respective cell motility, defined by a polynomial of degree 4 (n = 1,610).

Figure 1. Flow cytometry analysis and differentiation assays of mesenchymal stem cell (MSC) cultures. As shown by a representative sample, flow cytometry analysis revealed that MSCs expressed CD44 (A), CD90 (B), and CD105 (C) but not CD14 (D) (violet line with gray area indicates control; red line with white area indicates sample). (E): Differentiation of induced (positive control) and noninduced (negative control) MSCs. The adipogenic phenotype was determined by Oil Red O staining (top row, scale bar = 200 μm), the chondrogenic phenotype by Alcian blue staining (bottom row/left), and the osteogenic phenotype by von Kossa staining of mineralized matrix deposition (in black; bottom row/right). Abbreviations: Ctrl, control; FITC, fluorescein isothiocyanate; Neg., negative; Pos., positive.

Figure 2. Cell tracking of mesenchymal stem cells. Representative micrographs (n = 70) of slow-moving (A) and fast-moving (B) populations of mesenchymal stem cells recorded for a 24-hour period. Each colored line represent the track of a single cell (scale bar = 200 μm). Insets: Enlarged view of the tracked cells (scale bar = 100 μm). (C): The distribution of all single cell tracks was plotted versus the number of cells with the respective cell motility, defined by a polynomial of degree 4 (n = 1,610).

Alcian blue precipitation assay for chondrogenesis, and (c) Oil Red O staining measurement for adipogenesis. The collected results were directly compared with the distribution of cells (represented in the graphs as single dot) within a population (represented in the graphs as a row) according to the cell motility. Pooling all cells for analysis revealed a distribution pattern favoring middle speed cell motility and correlating with the respective osteogenic (Fig. 4A), chondrogenic (Fig. 4B), and adipogenic (Fig. 4C) differentiation potentials. Specifically, the analysis of all samples using penalized spline smoothers showed that the highest osteogenic and adipogenic differentiation output was produced by cells with a motility of 0.5 mm/day, and the cells with a motility of 0.7 mm/day had the highest chondrogenic potential.

Cell Tracking Versus Population Heterogeneity

We measured the average motility and variability in MSC populations to determine the relation between motility and heterogeneity (Fig. 5). The populations with the highest heterogeneity of cell speeds had a cell motility mean of 0.33–0.61 mm/day, and the populations with a mean outside this range were more homogeneous (i.e., the prevalence of slow cells in the population less than 0.33 mm/day and the prevalence of fast cells in the populations greater than 0.61 mm). An analysis of all populations using penalized spline smoothers confirmed this trend but only for the populations with an average motility faster than 0.33 mm/day. At less than 0.33 mm/day, only a single population could not be considered representative of the group. Also, enrichment of the senescent cells (very slowly moving cells; not on the plot) was seen. We could not find any direct relation between the grade of speed...
compared with the fast-moving cells, which had three times fewer senescent cells. The average speed cells had spindle-shaped morphology, an average cell size, and an average proportion of senescent cells. However, some spindle-shaped cells were moving quickly enough to be included in the fastest group. This discrepancy resulted from interesting changes in morphology—some cells alternated between round and spindle-like shapes. Similar results in cell morphology [11, 25] and senescence [15] were obtained from other studies of MSC populations.

The relationship between MSCs senescence and potency has long been studied. In our previous study, we proposed a senescence score in which combined markers provided reliable quality control of MSCs, depending not exclusively on the mechanistic passage number [24]. Similarly, many other reports have compared the composition of MSC populations with differentiation potential to find an assay that could predict the potency a priori. These methods were based on the growth rate and proliferation [14, 26], a colony-forming assay [27], the expression of age-predictive genes [28], and the morphological [13] and immunocytochemical [25] characteristics of MSCs. Almost all of these assays require at least several days to perform. To our knowledge, before our report, cell motility has never been used as a tool for dissecting the cell behavior of the individual cells in MSC populations. The advantages of this approach were the easy handling of cell cultures and the short time required to analyze the data compared with the methods listed. Cell motility could be also used in the future to develop predictive assays of the immunoregulatory activity of MSCs.

As expected, among the MSC populations, broad and unpredictable diversity was found in the respective differentiation outputs. Most interestingly, the cells with average speed possessed the highest differentiation potential compared with the slower and faster cells. This is a new confirmation of the Goldilocks principle in biology (i.e., the average population motility must fall within a “safe” margin to be applicable). However, the caveat was that the proportion of average motility cells within a population could not predict the differentiation output (i.e., populations enriched with average motility cells did not necessarily represent the best results). Any discrepancy between the parameters estimated at the single cell level and the whole population can be explained because either (a) our assay could not discriminate between cells with different multilineage potential (i.e., unipotent MSCs were evaluated as tripotent MSC, with a consequent alteration of the data), or (b) the populations with a median cell motility of 0.5 mm/day (the speed corresponding to the peak in cell differentiation performances) were characterized by higher cell heterogeneity and, thus, including slower or faster subpopulations that might inhibit differentiation. We observed that the MSC populations enriched with slow or fast cells were more homogenous (had less variation) in the distribution of cell speeds (e.g., had many slow or fast cells within the same population)—most importantly, these cells also had a lower differentiation potential. Thus, we speculate that the dynamics occurring between MSC subpopulations might play an important role in stem cell commitment. The limitation of the present study was that we did not separately analyze the subpopulations derived from the same bone marrow sample. Future studies should aim to dissect the heterogeneity considering the clonal subpopulations within a sample to determine whether the subpopulations resemble the parental population or have discrete speed patterns. We did not assay the grade of cell maturity (surface receptor analysis) or cytoskeleton composition of the cells during cell tracking because of concerns that any antibody labeling might interfere with the cell motility.

Figure 3. Mesenchymal stem cell motility correlated with cell morphology (A), cell size (B), and cell senescence (C). Slow-moving cells were characterized by a larger area and/or surface and a higher proportion of senescent cells compared with fast-moving cells. Cells with a smaller and round cell body moved than did the larger ones, and spindle-shaped cells had an average speed (mean ± SD; *, p < .05). Abbreviation: β-Gal, β-galactosidase.

DISCUSSION
In the present study, we found that the in vitro motility of MSCs in monolayer cultures could be connected to variables such as cell size and senescence grade and correlated with the intrinsic potential of the subpopulations to differentiate to osteogenic, chondrogenic, and adipogenic lineages. MSC populations were isolated from the bone marrow of 23 individuals at various in vitro passages (from P3 to P11) to include in the study several and diverse MSC populations, such as would be realistically encountered in daily MSC applications. Our assay was based on the life imaging of MSCs for a 24-hour period, followed by the tracking of 70 random cells per sample. We specifically recorded the cells in the first 24 hours after attachment because, within that period, the cell division rate is very low; thus, the division time interfered minimally with our tracking measurements. We found that mitosis occurred between approximately 60 and 90 minutes; however, within this period, the cells do not stop moving because of cytokinesis. Thus, we also included the few dividing cells as representative of the whole cell population. From our observations, we could categorize the MSC populations into three groups according to their cell motility pattern: slow, average, and fast. We observed that the MSC morphology and size correlated with the motility performance, with small and round cells moving faster than large and flattened cells, which, according to the published data and our experience, tend to be senescent cells [24]. We also showed that slow-moving cells had a greater proportion of senescent cells, identified by elevated senescence-associated β-galactosidase activity within the sample, compared with the fast-moving cells, which had three times fewer variability of the MSC populations and their differentiation potential (data not shown).

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CONCLUSION

The interdonor variability and clonal heterogeneity of MSC populations are major challenges to developing effective MSC-based therapies. Using live imaging and motility tracking of MSCs in vitro, we have developed a method to predict the intrinsic differentiation potential of a given MSC sample. Our novel approach revealed that cells with average motility possessed the highest differentiation potential compared with the slower and faster cells. However, the MSC samples enriched with the average motility subpopulation were also the most heterogeneous, which might influence the differentiation outputs. From a translational viewpoint, the present study proposes a new time- and cost-saving approach toward quality control for the production of large quantities of MSCs with predictable differentiation potential for research and/or clinical applications.

ACKNOWLEDGMENTS

This work was supported by the Swiss Paraplegic Foundation and Swiss National Foundation Grant CR3I3_140717/1.

AUTHOR CONTRIBUTIONS

A.B.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; A.G.: data analysis and interpretation and final approval of manuscript; M.G. and B.G.: collection of data and final approval of manuscript; M.B. and T.P.: provision of study material, final approval of manuscript; J.S.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript, administrative support.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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Figure 4. Potential of mesenchymal stem cells (MSCs) undergoing osteogenic, chondrogenic, and adipogenic differentiation was plotted against cell motility. Differentiation was measured by calcium accumulation (osteogenic), glycosaminoglycan accumulation (chondrogenic), and Oil Red O absorbance (adipogenic). Each row (70 cells) represents a MSC population. The open circles represent single cells; the line, the penalized spline; and the gray area, the confidence band. Abbreviation: GAG, glycosaminoglycan.

Figure 5. The mean motility of each population was compared with the coefficient of variation (SD divided by the mean). Each circle represents a cell population derived from one donor. The homogeneous population (low variance of cell speeds within the population) are located at the bottom of the graph, and the heterogeneous populations (high variance of cell speeds within the population) are at the top. The most heterogeneous populations are located within the interval of 0.33 and 0.61 mm/day. The line represents the penalized spline, and the gray area represents the confidence band.

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