Differentiation Potential of Mesenchymal Stem Cells of Different Origin

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We compared differentiation potential of mesenchymal stem cells originating from human bone marrow, fatty tissue, thymus, placenta, and skin. The cells were characterized by differentiation into adipocytes and osteoblasts. Mesenchymal stem cells from different sources exhibited different differentiation potential, manifesting by the rate of differentiation and percentage of differentiated cells. Presumably, differentiation of mesenchymal stem cells derived from different tissues can differ due to the presence of progenitor cells of different types.

Key Words: *bone marrow; thymus; fatty tissue; placenta; skin*

Mesenchymal stem cells (MSC) are a prospective object for the use in cell therapy and are therefore intensely studied by many research groups. MSC are characterized primarily by expression of surface markers and differentiation potential. MSC express a series of specific markers (CD44, CD90, CD105, CD13, *etc.*) and should differentiate into cells of mesodermal origin: adipocytes, osteoblasts, chondrocytes. Due to prospective clinical application, MSC from different sources are actively studied. For example, MSC isolated from the bone marrow, fatty tissue, placenta, umbilical blood, pancreas, dental pulp, and synovial fluid were characterized [3-7,10,11,13]. On the other hand, comparative characteristics of MSC are scanty; just few publications present comparison of bone marrow and fatty tissue MSC [2,3,6], bone marrow and placental [12], bone marrow and dental pulp MSC [7]. Comparative characteristics of bone marrow, periosteum, synovial fluid, skeletal muscle, and fatty tissue MSC are more detailed [9].

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We previously compared human MSC isolated from several sources by expression of some surface markers using flow cytofluorometry [1]. Comparative morphological and proliferative analysis of MSC was also carried out. According to our data, the characteristics of MSC are similar and the cells in fact do not differ by their morphology and expression of markers. For more detailed characterization of MSC their differentiation potential should be evaluated.

Here we compared differentiation potential of MSC isolated from 5 sources: human bone marrow, fatty tissue, thymus, skin, and placenta.

MATERIALS AND METHODS

Human bone marrow, fatty tissue, placenta, skin, and thymus were analyzed. Bone marrow, fatty tissue, and skin were collected from healthy adult donors (13-58 years, mean age 25 years), fragments of the placenta were collected during normal labor, and specimens of the thymus were collected from stillborn fetuses. All donors and/or their relatives gave informed consent to the use of the material for research purposes.

Bone marrow (10-15 ml) was collected by puncture of the ileac bone under local analgesia.

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Fatty tissue (3-5 ml) was collected through a small incision in the navel fossa, also under local analgesia. Skin fragments (1.0-1.5 cm^2) were collected from patients during surgical interventions. Cells from several sources (fat, skin, placenta, thymus) were collected and cultured under identical conditions. The bone marrow was processed by classical methods; mononuclear cell fraction was obtained by centrifugation in Ficoll density gradient (1.077).

The bone marrow was diluted in an equal volume of PBS (Gibco), applied onto Ficoll (Pharmacia) solution, and centrifuged at 400*g* for 30 min at 10°C. The median cell fraction was collected, washed in PBS, centrifuged at 200*g* for 10 min at ambient temperature. Erythrocytes were lyzed in 160 mM NH_4Cl. The cells were washed and inoculated ($1\times10^6/cm^2$).

Tissue fragments were washed in PBS, crushed, placed for 30 min into 0.075% collagenase solution (Invitrogen, type 1 for skin and fat, type 2 for thymus, type 4 for placenta) at 37°C. The enzyme was inactivated with DMEM with 10% FBS (Gibco), centrifuged at 200*g* and ambient temperature for 10 min, washed several times in PBS, and, if necessary, erythrocytes were lyzed in 160 mM NH_4Cl. Fragments of lyzed tissues were removed by filtration through Millipore nylon filters (100-μ pores). The cells were inoculated similarly as bone marrow cells ($1\times10^6/cm^2$). The medium was replaced after 24 h. Culturing was carried out in DMEM (Gibco) with low glucose content (1 g/liter) with 10% FBS (Gibco), 2 mM glutamine, 1% penicillin and streptomycin. The cells were cultured under standard conditions (37°C and 5% CO_2). The medium was replaced every 3-4 days. After 80% confluence was attained, the cells were removed with 0.25% trypsin/EDTA and inoculated at a concentration of 6-10 cells/cm^2.

After 2 passages MSC isolated from all sources were inoculated at a concentration of 15-20 cells/cm^2 and cultured in DMEM (4 g/liter glucose, 2 mM glutamine, 1% antibiotic/antimycotic, 10% FBS) for 3 weeks in the presence of the following reagents: 0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, 10 μM insulin, 200 μM indomethacin (adipogenic differentiation); 0.1 μM dexamethasone, 50 μM ascorbate-2-phosphate (osteogenic differentiation).

After 3 weeks of culturing differentiated cells were washed twice in PBS and fixed. Adipocytes were fixed in 4% formaldehyde for 60 min at ambient temperature and then incubated in 0.5% Oily Red stain for 20 min. Osteoblasts were fixed in glacial 70% ethanol for 1 h and stained in Alizarin

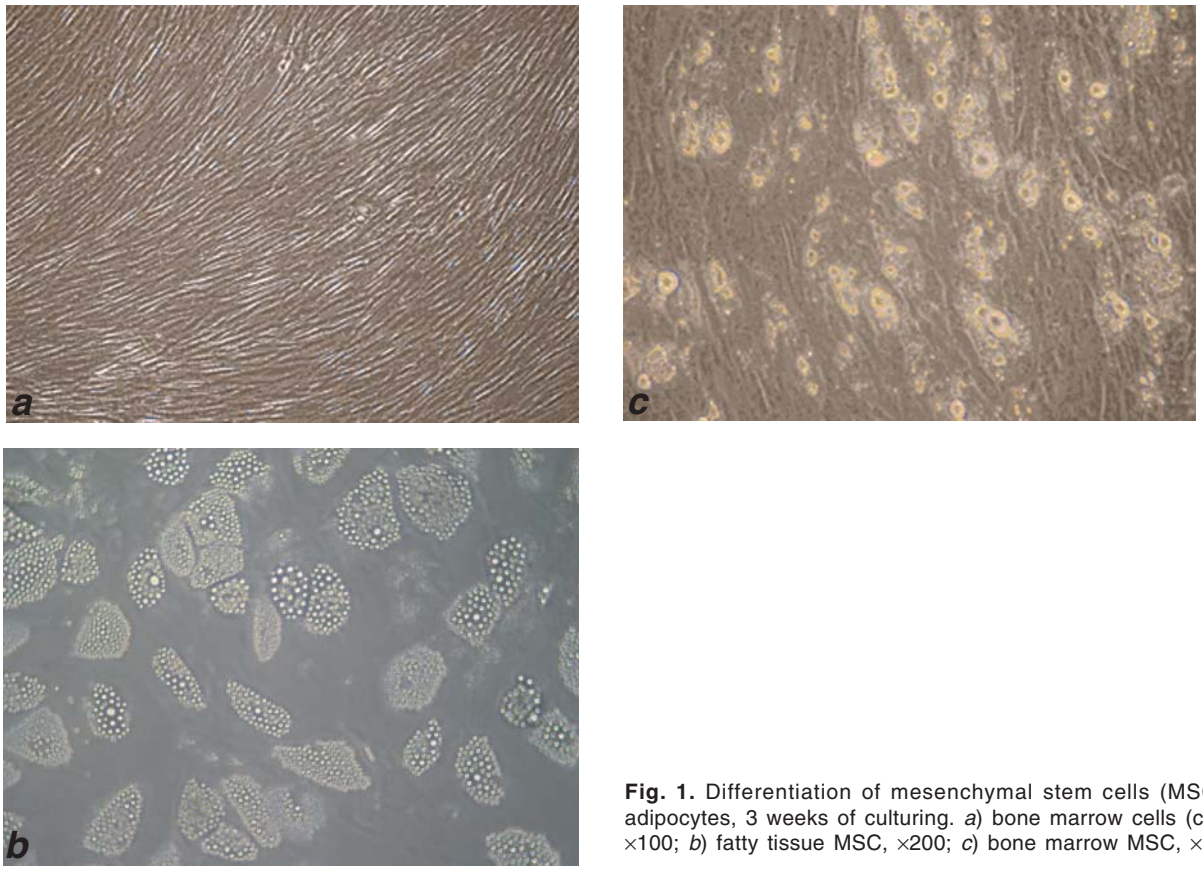

Fig. 1. Differentiation of mesenchymal stem cells (MSC) into adipocytes, 3 weeks of culturing. *a*) bone marrow cells (control), ×100; *b*) fatty tissue MSC, ×200; *c*) bone marrow MSC, ×100.

Red (40 mM, pH 4.1) for 10 min. Stained cells were photographed under a phase contrast microscope (Olympus CK 40M) and ImageScope Lite software.

RESULTS

The morphology of cells from all tissues changed during differentiation into adipocytes and osteoblasts. The developing changes in the cultures were easier to detect and evaluate visually during differentiation into adipocytes, because the cells started accumulating lipids.

The cells derived from fatty tissue first started differentiation. The first lipid inclusions appeared in the cultures after 3 days. After 1 week 30-40% fatty tissue MSC were differentiated. By this term the first differentiated cells appeared in cultures derived from the thymus. Skin MSC started differentiation after several more days. Bone marrow and placental MSC started differentiating much later, the first lipid incorporations appeared in these cultures only during week 2 of culturing. By this time 90% fatty tissue MSC, 30-40% thymic MSC, and 25-30% skin MSC were differentiated (Fig. 1, *a-c*).

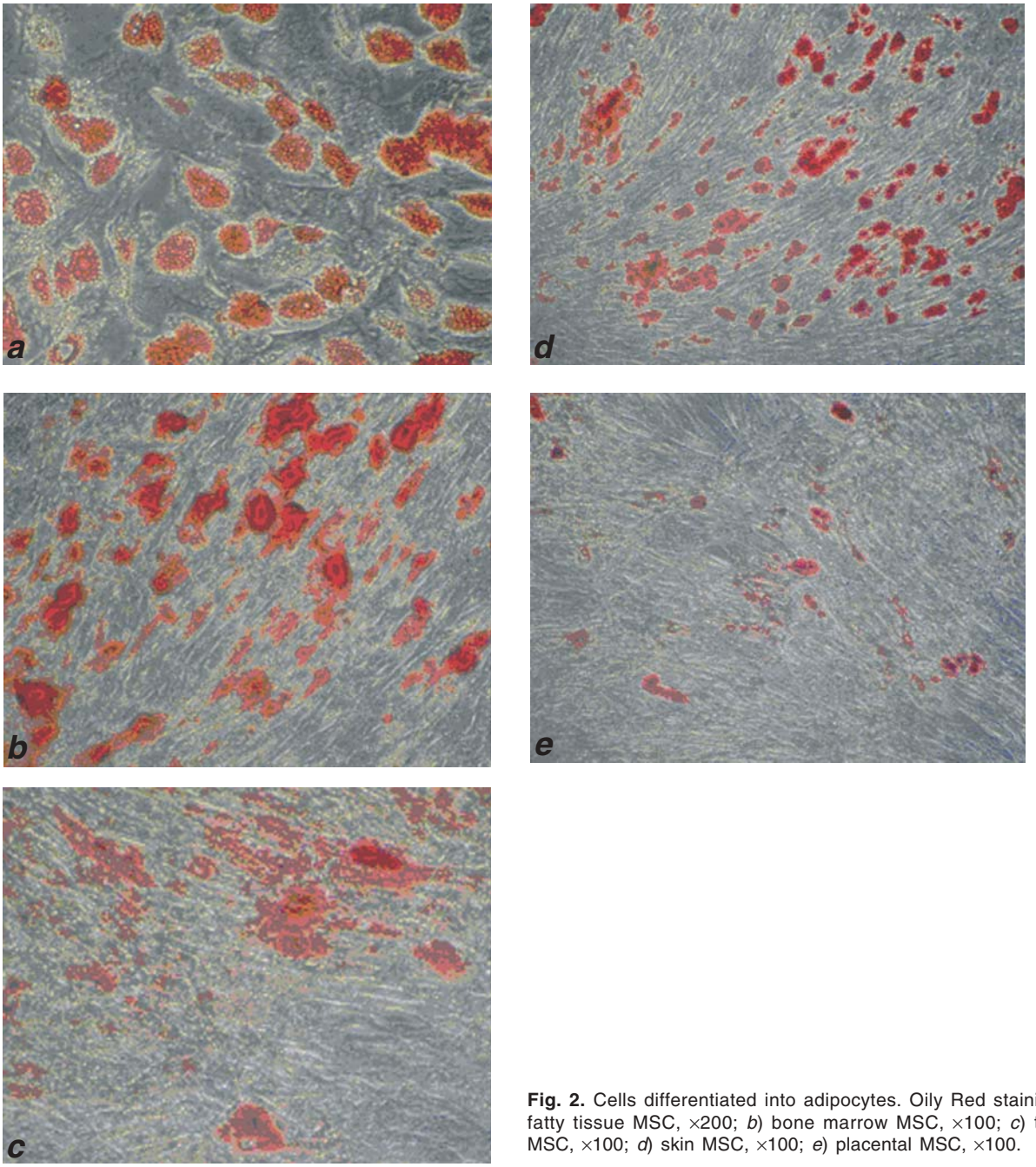

Fig. 2. Cells differentiated into adipocytes. Oily Red staining. *a*) fatty tissue MSC, ×200; *b*) bone marrow MSC, ×100; *c*) thymic MSC, ×100; *d*) skin MSC, ×100; *e*) placental MSC, ×100.

Bone marrow MSC significantly accelerated differentiation during week 3 of culturing and the percentage of differentiated cells surpassed that in cultures from the thymus and skin. By the end of week 3 in culture 90% fatty tissue MSC, 60-70% bone marrow MSC, 50-60% thymic MSC, 40-50% skin MSC, and 30-40% placental MSC were differentiated into adipocytes (Fig. 2, *a-e*).

Visual observation of osteogenic differentiation was difficult. We noted changes in cell morphology in all cultures: the cells acquired a more round shape with specific accumulations. By the end of week 3 the cells in all cultures were stained with Alizarin Red (Fig. 3, *a-e*). Bone marrow MSC most actively differentiated into osteoblasts; fatty MSC ranked second by staining intensity. Skin, thymus, and placental MSC contained approximately the same content of cells differentiated into osteoblasts.

It is obvious that the differentiation potential of MSC isolated from different tissues can be different. According to previous findings [9], the differentiation potential of MSC derived from the fatty tissue, bone marrow, periosteum, synovial fluid, and skeletal muscles differed significantly.

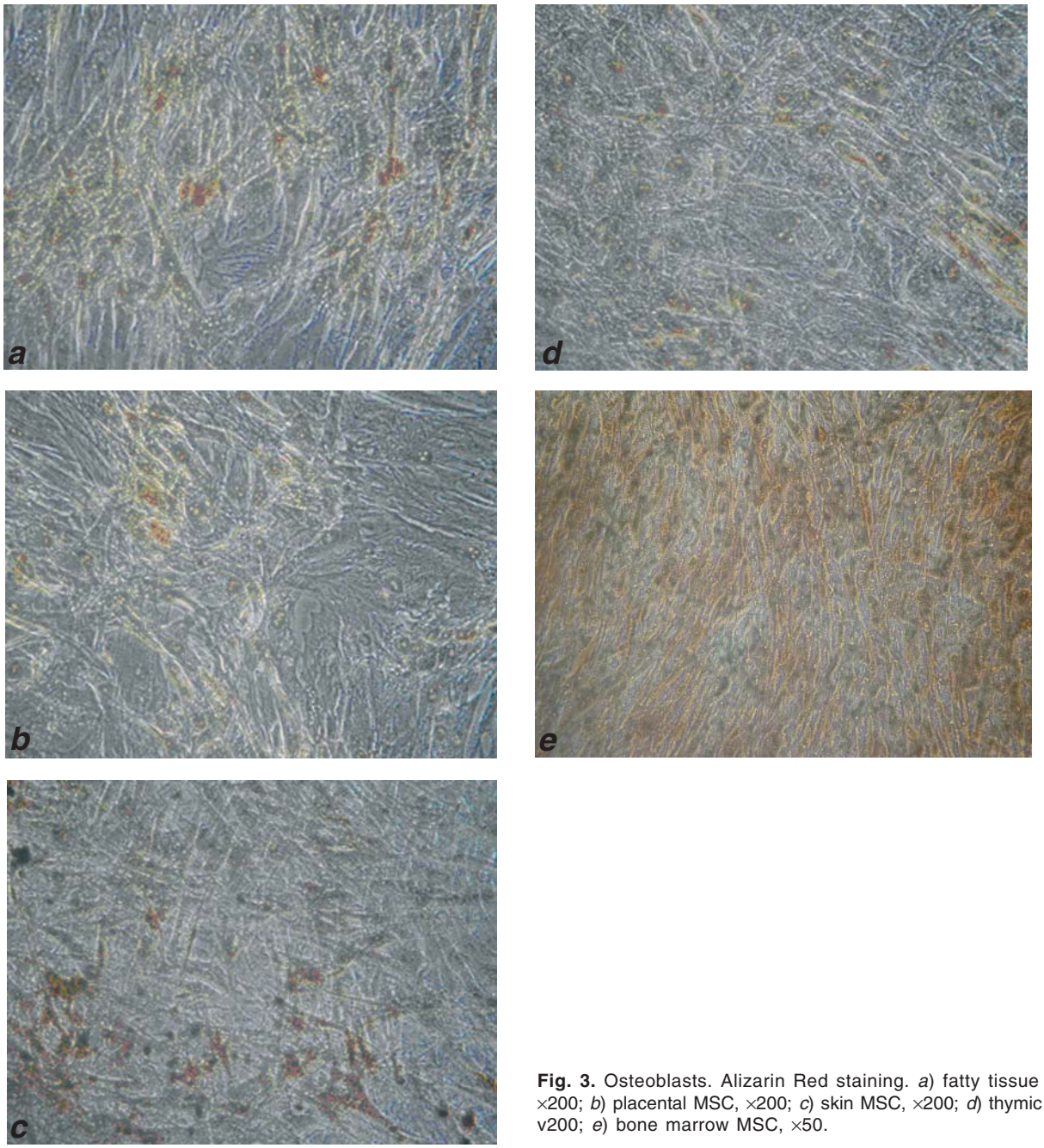

Fig. 3. Osteoblasts. Alizarin Red staining. *a*) fatty tissue MSC, ×200; *b*) placental MSC, ×200; *c*) skin MSC, ×200; *d*) thymic MSC, v200; *e*) bone marrow MSC, ×50.

The differentiation of MSC is very important for clinical application. It seems that the data on the differentiation potential of MSC and the use of MSC from different sources (autologous and/or allogenic) will help to determine optimum combination of cells for disease treatment. MSC from different sources differ by proliferative activity and immunosuppressive effects [9]. Some reports indicate that autologous bone marrow MSC from patients with arthritis possess lower capacity to differentiate into chondroblasts [8]. It seems that in these cases autologous cells for transplantation should be supplemented by donor material in cell therapy; which specifically donor material should be used — is a question of further investigation. We plan to extent the range of our studies on characterization of individual MSC types of adult and fetal human cells, with consideration for gestation period.

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