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MULTIPOTENT MESENCHYMAL STROMAL CELLS ISOLATED FROM SUBCUTANEOUS FAT OF MAMMALS FOR THE STUDY OF *Sarcoptes Scabiei/mange* in vitro

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Abstract

Sarcoptes scabiei/mange is a small, roundish, pale gray mite that lives in the epidermis of mammalian skin and causes scabies. Despite the fact that the mite biology is well studied and its interaction with the host is intensively investigated, the data analysis demonstrates the absence of a cell culture on which the *Sarcoptes scabiei/mange* could multiply in vitro. This hinders the development of modern effective methods of diagnosis of this disease, the study of the immune response after infection in order to create vaccines and evaluate the effectiveness of the use of drugs for the clinical treatment of mite-borne disease. In this regard, the search for a cellular system that would maintain the viability of *Sarcoptes scabiei/mange* in vitro is particularly relevant. We describe cellular systems represented by multipotent mesenchymal stromal cells (MMSCs) that can be used for these purposes. MMSCs were isolated from subcutaneous adipose tissue (SAT) of cattle and humans. Biopsies were taken from healthy donors without clinical form of scabies. In MMSC of cattle and human, isolated from SAT scabies mite (*Sarcoptes scabiei/mange*) was found. The MMSCs isolated from cattle were the most contaminated by mites: of 13 MMSC cultures on the 2nd passage 9 cultures (or 69 %) were contaminated. For human MMSCs, contamination with mites was only found in one culture among 6 cultures obtained (16 %). The mite was found in the form of small dark spherical extracellular components, not characteristic for MMSCs, the number of which increased during culture. A characteristic feature of scabies mite reproduction in MMSC culture isolated from both cattle and human SAT was the formation of nests in the form of clusters. In the culture, we identified presumably larvae and nymphs of the mite. During subculturing within 3 passages, the contaminated cattle and human MMSCs retained the same vitality as not contaminated, but the monolayer formed more slowly, i.e. on days 9-10 vs. day 7 in the control groups. After freezing the samples in which the mite was found, and storage in a Dewar vessel in liquid nitrogen for a week, followed by defrosting, it was found that the mite retained viability in all samples and well tolerated freezing-thawing. After thawing, the mite was detected on the 2nd passage in 7 of 7 analyzed MMSC cultures from cattle SAT and in the only MSCS culture derived from human SAT in which the mite contamination was found (100 %). Thus, it is shown that the MMSCs isolated by us from cattle and humans SAT, may represent a promising cellular system for studying in vitro *Scabiei scabieis/mange*. Possible contamination of MMSCs from SAT with scabies makes testing of these cultures mandatory in case of their use in cellular technologies.

Keywords: multipotent mesenchymal stromal cells, subcutaneous adipose tissue, cell culture, *Sarcoptes scabiei/mange*, culturing, freezing

Itch mite (human itch mite *Sarcoptes scabiei*/animals itch mite *S. mange*) is an intradermal parasite of many mammals including domestic and farm animals. *Sarcoptes scabiei/mange* is an obligate ectoparasite which lives and reproduces itself inside the host epidermis [1, 2]. The mite destroys the host's cells both mechanically and by secreting the cytolytic components. These cytolytic components and the antigenic substances, feces or eggs of mites cause the immunopathogenic reactions [3]. The primary clinical sign is intense itching; later,

the cellular lesions in the skin may appear. Depending on the host immunopathological status, the symptoms, as well as the intensity, dissemination and course of the disease can vary greatly. The mange is widespread throughout the world and causes the significant decline of animals' life and in some cases leads to their death [4]. *S. mange* of farm animals causes the significant economic damage to the livestock sector, especially in pig breeding.

Despite the significant progress in understanding the mite biology and its interaction with the host, much is still unknown [5, 6]. For a long time, there was a discussion whether *S. scabiei* is a single species or it should be divided into several species specific for each host organism, but there are still no genetic evidences of the existence of several species or subspecies of this mite.

The analysis of literature data indicates the lack of in vitro studies of the mite. Saliva, molting enzymes and hormones, feces and nitrogenous substrates secreted by the mite into the extracellular fluid surrounding the epidermis and dermis cells can influence these cells including keratinocytes, fibroblasts, macrophages, mast cells, lymphocytes, Langerhans cells, dendritic cells and endothelial cells. The results of the series of studies carried out, in particular, on mononuclear and dendritic cells [7, 8], keratinocytes and fibroblasts [9, 10], and endothelial cells [11, 12] showed that the extract made of the bodies of the *S. scabiei* mites modulates the secretion of cytokines by human keratinocytes and fibroblasts in cell culture. Thus, the addition of the extract when cultivation of human epidermal keratinocytes leads to the significant intensification of the secretion of interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF). In addition, the substances contained in this extract stimulated the increase in the secretion of interleukins 6 and 8, as well as of VEGF in culture of human skin fibroblasts. The attempts to study the influence of cellular interactions between keratinocytes and fibroblasts when the cells are affected by scabies mites and their extracts in vitro with using a three-dimensional model equivalent to the skin are being made [13, 14]. The results show that cellular interactions play an important role in the host's response to the mites.

The non-existence of the cell culture in which the mite could reproduce itself in vitro restrains many studies including those on the development of modern effective methods for diagnosing this disease, studying the immune response after the contamination by the parasite and creation of vaccines [15-18]. Currently, acaricides are used [19] to treat the *S. mange* infection but these are expensive drugs, and besides they may pose the risk to the environment, food products, animal trainers, etc. The systematic use of acaricides leads to gaining by the mites of high resistance to them. The mite is considered to be resistant to a range of drugs [20-24]. In this regard, the particularly pressing matter is to find the cellular system which would make it possible to assess the effectiveness of anti-mange drugs in vitro.

In our study, for the first time ever, we discovered the fact of contamination by the itch mite of the multipotent mesenchymal stromal cells (MMSCs) extracted from the subcutaneous adipose tissue of mammals and described the cellular systems of MMSCs which can be used for maintaining the viability of *Sarcoptes scabiei/mange* in vitro.

The aim of the work is to show the possibility of using multipotent mesenchymal stromal cells for maintaining *Sarcoptes scabiei/mange* in vitro.

Techniques. The MMSCs were extracted from subcutaneous adipose tissue of bovine cattle (beef cattle) and humans. The biopsy samples were taken from healthy donors (without any clinical form of mange) from the accidentally found contamination by mite.

The cells were extracted according to the methods described by us previ-

ously [25)] and cultured in DMEM (Dulbecco modified Eagle's medium) with low (1 g/l) content of glucose (PanEco, Russia), 10% fetal bovine serum (FBS) (HyClone, Perbio Scientific, Belgium) and solutions (1×) of nonessential amino acids and antibiotics (PanEco, Russia). The final concentration of streptomycin in the medium was 50 μg/ml, penicillin concentration was 50 U/ml. The medium was changed every 4 days; upon reaching 90% of the monolayer, it was treated with 0.25% trypsin solution (PanEco, Russia) and the subculturing was continued at the cells density of $5\times10^3/cm^2$.

The morphology of cells and mites in culture was assessed visually using the inverted phase-contrast microscope (Carl Zeiss, Germany) with AxioVision Rel. 4.8 software (Carl Zeiss, Germany) for measurements.

The mite impact on the MMSCs contaminated by them was evaluated by the viability of cells and their rate of monolayer formation when subculturing (3 passages). The cell cultures of the same density relevant to each group but not contaminated with the mites were control samples. The cells viability was assessed by trypan blue staining (0.1% solution, PanEco, Russia).

The MMSCs was frozen according to the standard method in the cryoprotective medium with 10% dimethyl sulfoxide (DMSO) in gradual mode of cooling up to −70 °C at the rate of 1 °C per minute; then the ampoules were transferred into liquid nitrogen (−196 °C) and stored for 1 week. The cells were quickly defrozen in a water bath (37 °C) and the DMSO was immediately separated by low-speed centrifugation (1000 rpm, 5 min). The presence of mites in the samples subjected to freezing and defreezing procedures was evaluated after the formation of the cells monolayer.

Results. It is difficult to visualize *S. scabiei* in primary culture just after the extraction of cells from the tissue. We accidentally found a mite in the MMSCs extracted from subcutaneous adipose tissue (SAT) of cattle after the first subculturing. It was noted that in some samples of cell cultures extracted from SAT there are visible presence of extracellular components in the form of small dark spherical structures, which are not characteristic for MMSCs (Fig. 1, A) and the number of which had been increasing as time passes (see Fig. 1, B). When more detail viewing, both mites themselves (see Fig. 1, D) and their niduses formed by them in culture (see Fig. 1, C), with the accumulation of cells in these places have been found.

When subculturing, the MMSCs which have been contaminated by mites, for 3 passages maintained the same viability just like non-contaminated ones, but the monolayer was formed more slowly. Thus, in the control groups of cattle and human MMSCs, the monolayer was formed on day 7, and in the contaminated groups on day 9-day 10.

The cells extracted from cattle SAT samples turned out to be most contaminated by mites: altogether, 13 cattle MMSCs cultures were analyzed at the 2nd passage, of which 9 cultures (69%) were contaminated.

When analyzing the MMSCs from human SAT, only one culture of 6 obtained (16%) was found to be contaminated by mites. The finding out of *S. scabiei* in the culture of multipotent mesenchymal stromal cells extracted from human subcutaneous fat tissue is illustrated in Figure 2. The features we observed during the cultivation of mites in MMSCs of cattle SAT were noted also in the MMSCs of human SAT. The presence of mites was indicated by dark formations of spherical shape, which are not characteristic for MMSCs (see Fig. 2, A), which number had been increasing as time passes (see Fig. 2, B). When cultivation, the niduses (conglomerations) formed by mites in culture were seen (see Fig. 2, C, D). Separate mites have been found in the culture's medium (see Fig. 2, D).

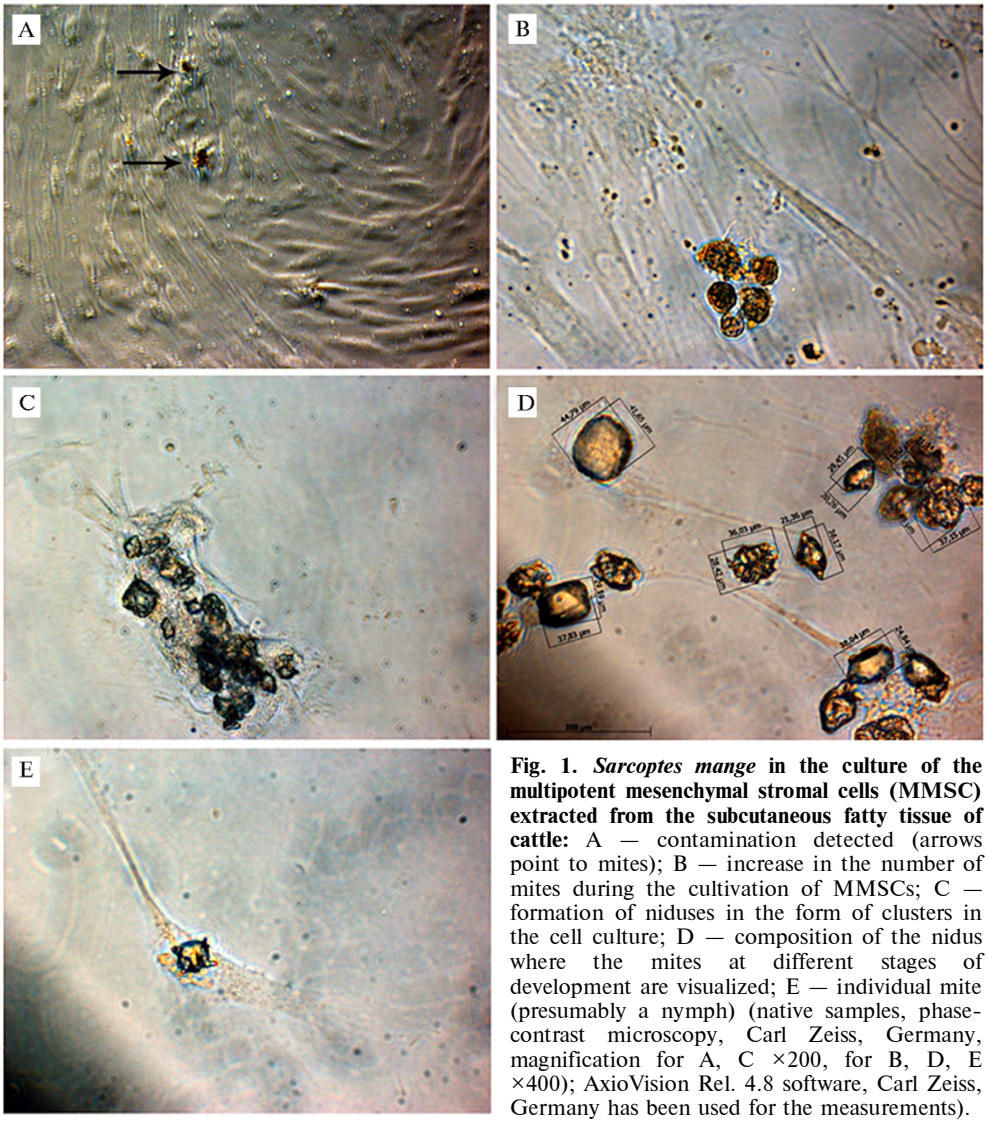

Fig. 1. ***Sarcoptes mange*** **in the culture of the multipotent mesenchymal stromal cells (MMSC) extracted from the subcutaneous fatty tissue of cattle:** A — contamination detected (arrows point to mites); B — increase in the number of mites during the cultivation of MMSCs; C — formation of niduses in the form of clusters in the cell culture; D — composition of the nidus where the mites at different stages of development are visualized; E — individual mite (presumably a nymph) (native samples, phase-contrast microscopy, Carl Zeiss, Germany, magnification for A, C ×200, for B, D, E ×400); AxioVision Rel. 4.8 software, Carl Zeiss, Germany has been used for the measurements).

When subculturing the MMSCs contaminated by mites, for 3 passages the cells maintained the same viability like non-contaminated ones, but they had been forming the monolayer more slowly. Thus, in the control groups of cattle and human MMSCs the monolayer was formed on day 7, and in the contaminated groups on day 9-day 10.

The causative agent of sarcoptosis (scabies), well known in veterinary medicine, are small, round-shaped mites of pale gray color. It is known that a male mite (length 0.23 mm, width 0.19 mm) is smaller than female mite (length 0.45 mm, width 0.35 mm). The eggs are large, oval, length 0.15-0.25 mm, have a two-layer shell. The mite ontogenesis includes the larvae and nymph stages[1-3]. In all investigated samples we have not find any eggs and adult mites. It can be assumed that they do not attach to the cells, but are in suspension. In the culture, presumably larvae and nymph of mites have been found (see Fig. 1, D).

The analysis of data presented in the scientific literature earlier demonstrates the absence of the fact of contamination of cell cultures by mites *in vitro*. The multipotent mesenchymal stromal cells (MMSCs) extracted from the stromal-vascularized fraction of subcutaneous fat of mammals have the properties similar to the properties of the MMSCs extracted from bone marrow. They are

considered to exhibit immunomodulatory properties and produce a number of key cytokines for maintaining the hematopoiesis in vitro [26, 27] that may influence on the maintenance of the mite viability in MMSCs culture. In this respect, the work [28] in which the reaction of *S. scabiei* var. *canis* to the fats (mixture of 21 lipids) being typical for the human skin epidermis has been assessed and 13 fatty acids and their derivatives attracting this mites at all stages of their development have been identified, is of interest. MMSCs in culture can be subjected to the spontaneous differentiation toward the adipogenesis [25] that also can explain our data.

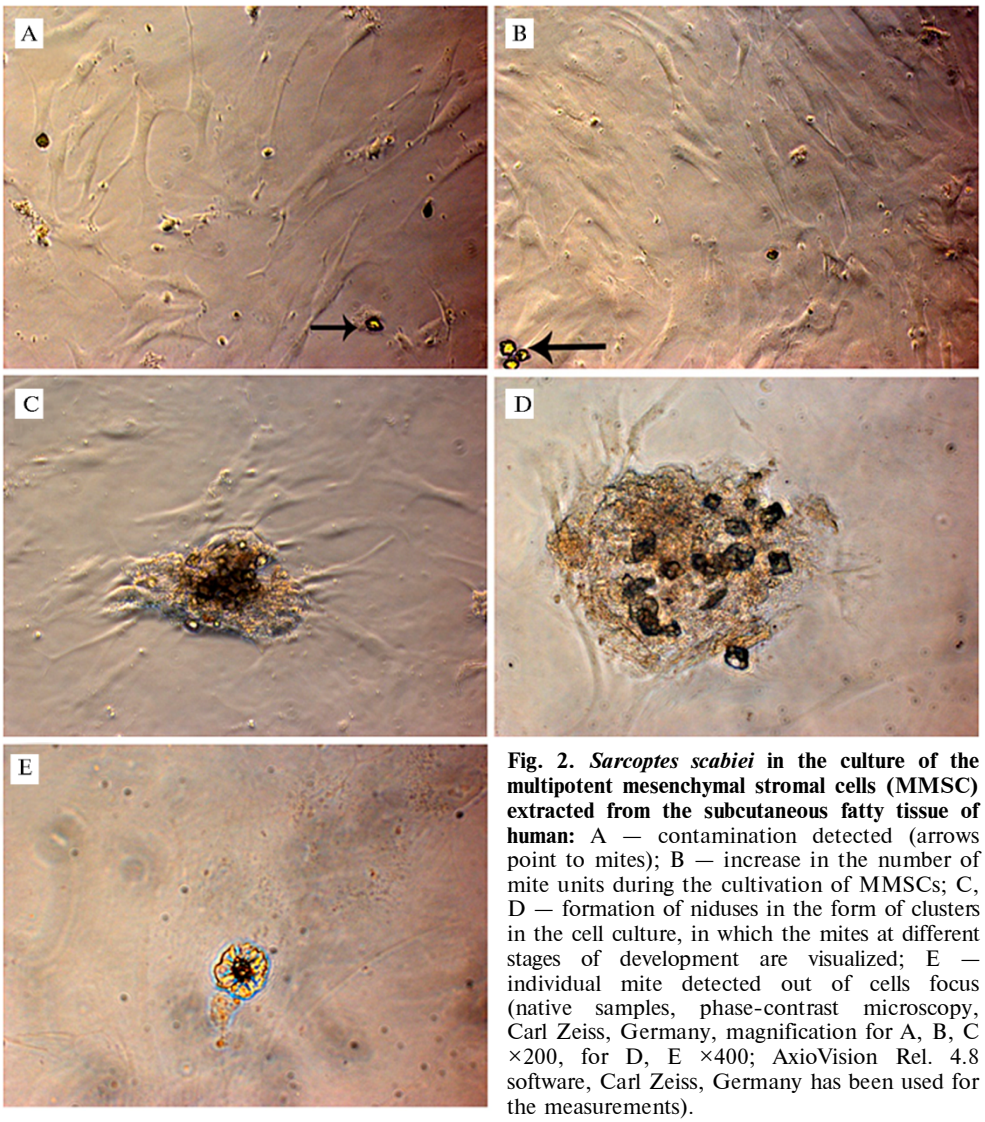

Fig. 2. *Sarcoptes scabiei* in the culture of the multipotent mesenchymal stromal cells (MMSC) extracted from the subcutaneous fatty tissue of human: A — contamination detected (arrows point to mites); B — increase in the number of mite units during the cultivation of MMSCs; C, D — formation of niduses in the form of clusters in the cell culture, in which the mites at different stages of development are visualized; E — individual mite detected out of cells focus (native samples, phase-contrast microscopy, Carl Zeiss, Germany, magnification for A, B, C ×200, for D, E ×400; AxioVision Rel. 4.8 software, Carl Zeiss, Germany has been used for the measurements).

It was of interest to assess whether mites retain their viability after the cryo-freezing of the cells culture. After freezing the samples in which mites have been detected, and storing them in a Dewar vessel in liquid nitrogen for 1 week followed by defreezing, the cells were cultured until the monolayer formation and the culture was assessed for the presence of *S. scabiei/mange*. After the defreezing, mites have been found in 7 of 7 MMSC cultures extracted from cattle SAT analyzed at the 2nd passage and in the single found MMSC culture from human SAT contaminated by mites (100%). Consequently, the mites retained the viability in all samples and tolerated freezing-defreezing well.

Due to the possibility to use the MMSC cultures extracted from human SAT in cell technologies, specialists should pay attention to the fact that, according to our data, such cultures can be contaminated by subcutaneous mites which are able to retain the viability while freezing and defreezing that indicates to the necessity of mandatory checking them for the presence of *S. scabiei*. The cultures contaminated by mites shall be discarded at the early stages after the extraction.

Thus, it has been shown that the multipotent mammalian mesenchymal stromal cells (MMSCs) extracted from the subcutaneous adipose tissue (SAT) of cattle and humans, can be the promising cellular system for studying *Sarcoptes scabieis/mange* in vitro. The discovery of the fact that MMSCs extracted from human SAT may be contaminated by mites makes the testing of such cultures mandatory in case of using in cell technologies.

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