ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)Biopolymers and Cell. 2022. Vol. 38. N 2. P 117–142
doi: http://dx.doi.org/10.7124/bc.000A78

UDC 577

MSC — What is it?

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The interest in MSCs is rapidly growing due to their possible and actual therapeutic properties. In terms of scientific studies and clinical application, primary attention is paid to the issues, related to the use of MSCs as a therapeutic means. Successful advance in this direction requires the understanding of the place of MSCs in the organism and those factors which caused the need for the evolutionary manifestation of the functions performed by special cells – MSCs. Thus, current notions about the mechanisms of the therapeutic effect of MSCs are considered here. These notions are compared to the experimental data about the phenomenology of therapeutic effects, registered after the administration of MSCs into the organism as a therapeutic means. The information on the special role of MSCs in the organism is analyzed, and the idea is formed about the essence of this special role. Based on the conducted analysis, the suggestion is made about directing the studies into enhancing the therapeutic effects of MSCs as a potentially highly efficient medicinal agent.

Keywords: MSC

Problem statement instead of introduction

The Earth is humanity's home. We are so used to this home that we do not notice the aggressiveness and destructive effect of our environment. At the same time, solar energy, reaching the Earth's surface, can break many chemical bonds. Oxygen, required for our breathing, is a powerful oxidizer, and the energy of its oxidation is inferior only to fluorine. Water is a universal solvent, unrivaled in its ability to dissolve and dissociate practically "everything" in the entire Universe. And we do not even ponder that the normal saline solution is actually stable covalent bonds in NaCl molecules, broken by water into their constituents Na^+ and Cl^-. And the expression "constant dropping wears away a stone" is a vivid demonstration of the fact that there are no substances insoluble in water whatsoever. There is only a degree of solubility. The pressure on a human body (≈ 2 sq.m.) is 20 thousand! kg. And this is true in everything. But there is an excellent "something" capable of ensuring our

existence and the possibility to enjoy life, taking no heed of the surrounding destructive processes.

Even more powerful energy-wise destructive processes occur within our organism. Different chemical processes constantly occur in each cell as an absolutely obligatory condition for its existence. They are mainly implemented via enzymes, and each is primarily well-studied separately. However, they are somehow spatially organized into mutually dependent chains in an actual cell. Each enzyme in them conducts a chemical transformation of the molecule (substrate), formed by the previous enzyme, which results in the occurrence of the following intermediate derivative that is the product for the following reaction, etc. [1]. For the biochemical reaction to occur, the enzyme takes some group of atoms of the substrate molecule, a specific molecule of this group, to the highly reactive state. Yet, if, as a result, the absolute spatial-temporal precision is breached, the reaction may occur not on the intended product but the neighbouring, closely located one. In addition to ensuring metabolism, there are many proteins, the catalytic activity of which ensures the movement of intracellular filaments, signalling, membrane permeability, RNA synthesis, protein synthesis, *etc.* (Table 1).

Table 1. **The main groups of cell proteins (UCB — MSC). (Modified from Feldman RE at al. 2005 [2] one UCB unit from a full-term delivery was isolated from the unborn placenta, transferred into culture, and their whole-cell protein fraction was subjected to two-dimensional electrophoresis (2-DE).**

Groups of cell proteins	Part of the total
Metabolism	25 %
Folding	14 %
Cytoskeleton	17 %
Signalling	8 %
Detoxification	3 %
Protein degradation	3 %
Transcription	5 %
Others	16 %
Transport	9 %

The intermediate products of enzymatic transformations are reactive to different degrees. No matter how perfect, highly accurate, specific, *etc.* biochemical reactions are, some formed reactive intermediate products go beyond the boundaries of active centres of "enzymatic activity". While contacting other stable but energetically weak bonds, they may form new "unsanctioned" combinations. If one accepts that at some moment of maximal activity of the cell, all this works in the mode of velocities and activities, determined and evaluated in laboratory studies *in vitro*, the mass of such products exceeds the mass of the very cell many times. The "average" cell contains 2.6×10^9 protein molecules [3]. Most of them have catalytic activity (Table 1). Let us accept that the number of proteins, the main function of which is to implement chemical reactions ("real enzymes") — metabolism, detoxification, degradation, transcription, *etc.*) — is 1×10^9 molecules. The average velocity of the reactions, determined *in vitro*, is 10 msec (100 revolutions per second). Let us take a lower average speed in the cell for our calculations: 10 revolutions per second. 10^9 (molecules of enzymes) $\times$ $\times$ 10 (revolutions per second) = 10^{10} intermediate metabolites per second. Almost all of them are highly reactive. If the relative mass of the reactive groups of intermediate metabolites is

taken as the mass of a classic highly reactive product H_2O_2 (34 Da), then, in dynamics (integrally), in 24 h each cell has the formation of:

34 Da (the mass of the relative reaction group) ×

× 10^{10} (the number of intermediate metabolites per second) ×

× 1.66×10^{-24} (a unit of molecular mass in grams) ×

× 8.64×10^4 (the number of seconds in 24 hours) ≈ 48.7×10^{-9} g, i.e. **48.7 ng.**

This is almost 500 times more than the mass of the "average" cell with the volume of 1,000 μm^3 which corresponds to ≈ 1 ng.

Regardless of the abstract nature of these calculations, the actual experimental data prove their actuality. The intensity of processes in the "average" cell in the real conditions is close to the ones, presented above [3]. And proteins with enzymatic activity make the main contribution. Yet, the intermediate products of metabolism are not the most highly reactive products. In case of breathing, the situation becomes even clearer, and even more rigorously counted in quantitative terms. The corresponding quantitative evaluation is presented in Fig. 1. Despite all the seeming unreality, the amount of peroxides and radicals, formed at the norm in a healthy organism during breathing, is equivalent to a 5-liter bottle of 40 % hydrogen peroxide (Fig. 1). For comparison, when applied to the skin, one drop of 3 % hydrogen peroxide (its pharmacy concentration) makes it white since it "burns" everything on the surface.

And no matter how perfect spatial-temporal organization of metabolism, detoxification systems, antioxidant systems, *etc.*, are, some highly reactive products, formed at the norm, cause unsanctioned side reactions, destroying the cell content. The evaluation of the degree of destruction, induced by highly reactive agents, avoiding the precise accuracy of processes and systems of defence is possible using

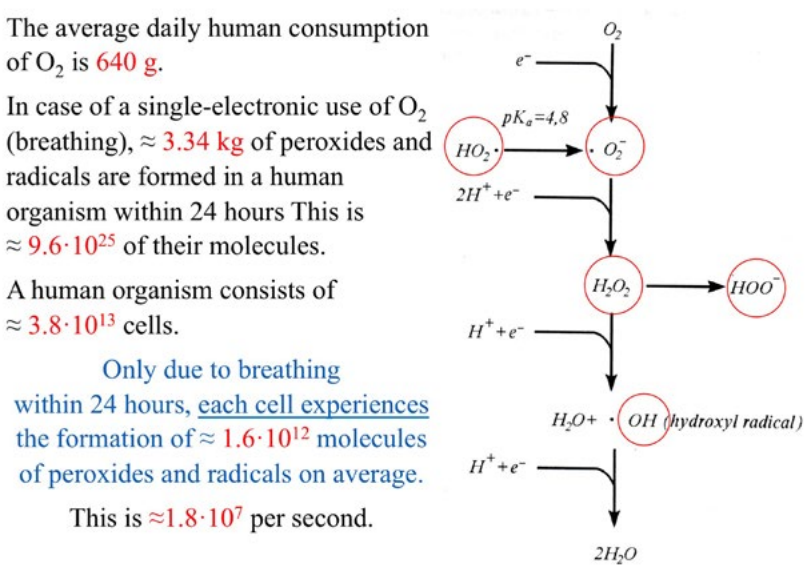

Fig. 1. Estimation of the possible number of intermediate reactive molecules formed during human respiration per day.

the analysis of quantitative processes of biosynthesis in the cell. 2.6×10^9 protein molecules in the cell are in their equilibrium state. On 6×10^6 ribosomes, 4×10^6 new protein molecules are synthesized every minute [3]. And the stable, normal existence of the cell requires the destruction of the very same amount — 4×10^6 protein molecules — every minute. This is the dynamics. In stable biosynthesis, there is the destruction of everything that functionally ceased to implement whatever it had been synthesized for. It means that the main bulk of the items under destruction is damaged. And these 4×10^6 damages are only a part of the damages that led protein molecules to the loss of their functional properties and made them non-working. With this dynamics of processes in the cell in 24 h, and with the abovementioned efficiency of synthesis/degradation, there will be more than a twofold change in the total mass of proteins: 4×10^6 (the number of protein molecules, synthesized per minute and thus the same number of eliminating ones) × 1,440 (the number of minutes in 24 h) / 2.6×10^9 (the equivalent total number of protein molecules) ≈ 2,2. This is the multiplicity of the turnover in the total mass of protein in the cell in 24 h or the index of intensity of destructive processes in the cell. But, besides protein, the cell also has other macro- and not only macromolecules, which will also be destroyed, restored, and replaced. Actually, the entire cell is in the self-supported equilibrium state in which the processes of self-destruction, self-synthesis, self-substitution, and self-restoration are constantly underway.

Potentially, the outside and inside destructive factors can destroy the cell in a matter of minutes. To avoid its implementation, each cell has numerous systems of prevention, protection, and restoration of all the structures, macromolecules, and their complexes on the molecular level. And whatever cannot be restored is replaced with the new. Yet, no matter how perfect these systems are, they let a considerable number of destructive agents in. As a result, there are numerous unrepairable damages in cells. The equilibrium state is breached, the percentage of "spoiled molecules" increases, and the cells perish. The total number of cells in the organism is very high but still limited. And the destructive processes in them are constantly going on. This should lead to the death of the whole organism very fast. And the verity of this fact is well known in the example of nematodes. In their well-studied representative, *Caenorhabditis elegans*, the division of cells stops prior to the embryogenesis completion after reaching the terminal number of 959 somatic nuclei. These are 959 cells that make up the nematode body. Its cells are not restored, and sometime later, after a short reproductive period, *C. elegans* dies. Thus, its species duration is about one month. Many species of metazoans usually live longer. As for humans, the species duration is a century.

A cell for the organism

For the death of cells (both imminent due to damages and scheduled) not to lead to the death of the organism, there is the all-organism system of protection, preservation, and restoration on the cellular level. It is implemented by three large groups of cells, which until recently have traditionally been called stem cells due to some of their properties, notable for true

stem cells. The first property out of this set is self-recreation. The second property is the possibility of asymmetric self-recreation. (In case of symmetric self-recreation, two identical cells with the same abilities of subsequent self-recreations are formed due to symmetric division. In case of asymmetric self-recreation, a stem cell is divided in such a way that one newly-formed cell remains a stem cell, whereas the other is deprived of this possibility and is terminal, regardless of its short or long way.) The third criterion of "stemness" is the ability to differentiate into different specialized cells of the organism. "True" stem cells have all three criteria and implement them in the organism. They ensure the equilibrium state of the number of cells of all types in the organism. The dynamics of this equilibrium demonstrates how powerful the destructive processes in a human organism are. The total turnover of cells in a human body is integrally estimated by the cumulative turnover value of $2–3 \times 10^{11}$ cells every 24 h [4]. Certainly, the velocity and completeness of the turnover are not the same in different tissues and organs, but it occurs every time and in every place (Table 2).

The visualization of the scale for the processes of cellular death/replacement of cells is presented in the following comparison:

"Our bodies collectively turnover about 200–300 billion cells every day. As part, epithelial cells of the gastrointestinal tract, which cover an area equivalent in size to a tennis court, are turned over every 4–5 days." [4].

The entire human body is endlessly changing as well. The total number of human cells is estimated as $\approx 3.8 \times 10^{13}$ [5]. Knowing the average daily turnover of cells, it is possible to calculate the summarized integral and annual dynamics: $2–3 \times 10^{11}$ (eliminated and equivalently formed a new cells in a human body every 24 h) × 365 (days in a year) / 5×10^{13} (total number of cells in a human body) ≈ 1.9–2.8 (yearly renewal multiplicity). The turnover of the content of cells is considerably higher. Let us take the dynamics of destruction and equivalent renewal of the whole content of cells, which is the same for their proteins (as presented above). It will amount to 2.4 (average renewal multiplicity per 24 h) × 365 (number of days in a year) ≈ 880.

Thus, human beings self-renew all their cellular macro- and even more micro-molecular content about 880 times a year and all their cellular content — almost twice a year (also, in a very average case). Due to ceaseless damages and reparation, the material which makes up DNA changes too. The exceptions are found in "lifelong non-dividing" cells of the brain and heart, the mechanisms of "eternity" (100 years without replacements) which are yet to be understood. Therefore, the human organism is its own "self-reproducer".

The second group of cells, ensuring the integrity and restoration on the cellular level (the second line of defence for the preservation and restoration of the cellular level) consists of cells that "self-reproduce" out of already differentiated ones in the form of immediately ready same cells without the need of subsequent differentiation which is not required for them, differentially self-reproducing cells.

The study of the self-reproduction mechanisms for this group of self-recreating cells in the organism is only starting. Their specificity is the ability of self-reproduction of the very

Table 2. **The average frequency of cell renewal in tissues. (According to Arandjelovic S.and Ravichandran K.S. [4])**

Organ or Tissue	Renew timing
Brain: nerve cells and neurons	practically do not renew themselves
Lungs	200 days
Skin (the epidermis)	10–30 days
Smooth muscles	1–1.5 months
Skeletal muscles	15 years
Liver	320–365 days
Heart: the cells of the cardiac muscle, the myocardium,	practically do not renew themselves
Stomach (the cells of the gastric mucosa, the epithelium)	2–9 days
Kidneys	270–300 days
Egg cells	do not renew at all
Intestines (epithelium cells)	2–4 days
Adipose cells	7.5–8 years
Skeletal bones	practically do not renew themselves
Blood: red blood cells, erythrocytes	4 months
Blood: immune cells, monocytes	2 days
Blood: immune cells, eosinophils	2–5 days

selves without the involvement of stem cells. In their crux, these cells remain a mystery. An example of these cells can be found in "tissue macrophages". According to all the properties, common to macrophages, they are typical macrophages. Yet they are not formed out of stem hematopoietic predecessors, rather they are in an independent population, which originates in a special lineage out of the yolk sac (Y.S.) and colonizes (!) an embryo, situated in that sac:

"…**macrophages in several tissues**, such as liver Kupffer cells, epidermal Langerhans cells, and microglia — cell populations that all can persist in adult mice independently of HSCs. These results define a lineage of tissue macrophages that **derive from the Y.S. and are genetically distinct from HSC progeny**." [6]. It is even more interesting because,

independently from the latter and after them, the hematopoietic stem cells are also formed as a lineage out of the yolk sac, also colonize the embryo, and also form tissue macrophages. But this is the "hematopoietic" part of tissue macrophages that (similar to all the blood cells) derives from the "hematopoietic" lineage, which originated in the yolk sac several days after the lineage that was forming autonomous tissue macrophages — the group of macrophages which, having become differentiated cells (macrophages), self-reproduce themselves, actually being stem cells for themselves [7]. And the macrophages, derived from the hematopoietic stem cells, are the terminal stage of differentiation and cannot self-reproduce. It is absolutely incomprehensible what and which functions require such autonomy. The degree of incomprehensibility is further aggravated by the fact that only "self-reproducing" macrophages are present in the brain. As for the peritoneum, there are only hematopoietic macrophages. And both the latter and the former are in the remaining tissues [8] (Fig. 2).

In addition to tissue macrophages, hepatocytes are self-recreating too. During embryogenesis, the liver was formed out of the predecessor cells and the hepatocytes were laid in it as those to be differentiated further on. And why, all of a sudden, they started possessing the unique ability of self-stemness, is not clear. Yet, it is noteworthy that even the macrophages of the liver (Kupffer cells) are also stem cells.

And an obvious question arises. If all these stem cells of both the first and second line do not have any predecessors anymore (some kind of even "more stem" cells which reproduce "merely stem" cells), and the damageability of the cells is very high, then how can all these stem cells exist "on their own" the entire species duration of a human being — 100 years? And as they actually do exist all this time and perform their functions, there must be something to provide for that. This "something" is

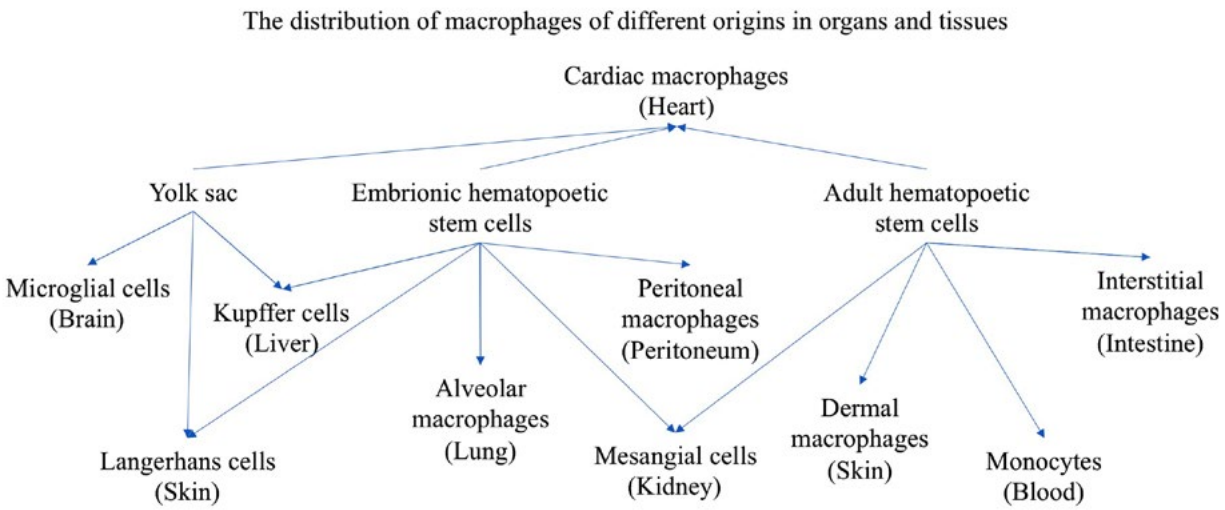

Fig. 2. Distribution of macrophages of different origin in tissues. (According to Shrivastava R. and Shukla N. [8])

the cells of the third line of the cellular level of protecting and preserving the organism.

A cell for a cell

The third group in the cellular link of preserving, supporting, and restoring the organism is MSCs. MSCs are heterogeneous populations of cells of mesenchymal origin. They were first discovered and isolated as a culture in the laboratory by Friedenstein in 1970 [9]. In their most generalized form, they are characterized as "colony forming units — fibroblasts", carry markers SD 73; S.D. 90^+; S.D. 105^+ and do not have SD 45^- [10]. At first, they were paid very little attention. Yet gradually, as their study progressed, the attention to them started increasing and since the early 1990s was getting more intense onward and upward. This type of cells turned out to be extremely relevant for the organism, very unusual, and interesting both as the object of fundamental studies and as an exclusively promising therapeutic means. It can be explained by the special functions of MSCs in the organism. If one summarizes the currently known experimental data and theoretical calculations, MSCs serve in the organism to support, preserve, protect, and restore all the cells of the organism tissues. But, in what way, according to which mechanisms MSCs implement this preservation, protection, and restoration in the organism is still, strictly speaking, unknown. Throughout the entire study of MSCs, the notions about the mechanisms of their action in the organism and the very cells have changed many times. It is reflected in the absence of their common full name and in on-going disputes about it. According to their determined biological functions, they were called "mesenchymal stromal cells", "mesenchymal stem cells", "mesenchymal multipotent cells", "mesenchymal stromal/stem cells", *etc.* One of the founders of the therapeutic application of MSC, A. Caplan, suggested moving beyond the tissue affiliation in their name. He suggested accepting the function of MSCs as the foundation for their name and calling them "Medicinal Signalling Cells" [11]. The attempts to unify the name of this type of cells have already been underway for 20 years. The markers are getting more complicated, the sources are introduced, *etc.* Yet everything changes unceasingly without achieving a commonly accepted nomenclature [11]. And now, to avoid their full name since it narrows their functions down, they are indicated with the commonly accepted abbreviation — MSCs. That is fine with everybody. Each understands their own under this abbreviation, and nothing is to be substantiated anymore. As for the biological phenomenon of MSCs, which, it is believed, is the foundation of their therapeutic properties, it can be divided into two large classes — "structural-management-supporting" and "transforming-preserving-restoring". Actually, MSCs are also capable of getting differentiated into specialized cells. And in the *in vitro* system, this is one of the tests for the investigated population of cells belonging to MSCs. Probably, it may also be implemented in the organism as an emergency situation. But only in some extreme cases. And the first notion about the functions of MSCs in the organism was their stromal function. It had both the structural component (supporting the specialized cell) and the management activity (ensuring the obligatory implementation of specialized functions by the specialized cell) [12] (Fig. 3).

Yet, the study of these properties of MSCs did not gain momentum though it was very interesting both as a phenomenon and as therapeutic potential. The main attention was paid to transforming-preserving-restoring properties. There have been numerous studies in this direction. They have mainly been conducted, coming out of classic views on the action of MSC. In general terms, these come down to distant and contact impact on MSCs on the immune system cells, which leads to the implementation of their therapeutic effects. Yet the direct immediate effect of MSCs on other, non-immune, cells is paid secondary attention.

The distant effect is made by a wide pool of all the types of biologically active components of the cell. Their unstructured constituent mainly contains different signalling molecules of all the classes — cytokines, chemokines, lymphokines, *etc.* At first, this very general pool of extracellular "biologically active", soluble products was attributed the therapeutic effect of MSCs [13]. But the notions were gradually changing. And five years ago, they started attributing the main effect of MSCs as a therapeutic agent to the structured constituent of the extracellular products. The structured constituent of the distant effect of MSCs consists of microvesicles, greatly differing in their sizes, origin, composition, and functions. By their structure, the microvesicles are spheric "basins", confined from the environment by the membrane, inside which the "content" is situated. In terms of sizes, the pool of vesicles fluctuated from 10 nm to 1,000 nm. Generally, the microvesicles are formed in two ways. One is budding from the cytoplasmic membrane. In this case, they are carrying its markers, receptors, ligands, *etc.* The other way is the formation of microvesicles inside the cell, after which they go through the membrane into the environment in this "ready" form. As for the composition, everything presents in the cell itself is found in the microvesicles. Certainly,

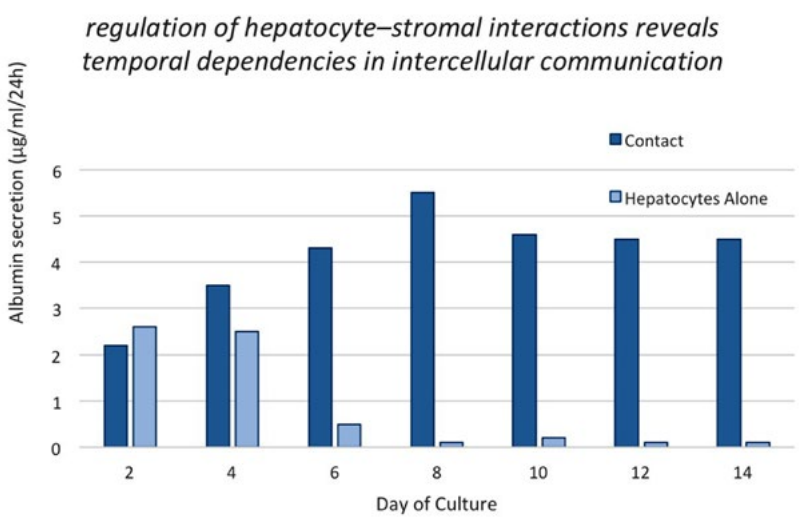

Fig. 3. When hepatocytes are transferred to the *in vitro* system, they, hepatocytes alone, quickly stop performing their primary biosynthetic function — albumin secretion. Yet, if stromal cells are present with them, the albumin secretion goes on for a long time. (Accordind to Hui E.E., Bhatia S.N. [12])

this "everything" is not in one vesicle, but somehow distributed among the entire pool of microvesicles. Yet summarily, this is truly "everything" — all the types of signalling molecules, including micro-RNA, ribosomes ready for work, informational RNA, DNA of the genome, and cytoplasm fragments. As a result, the microvesicles are capable of ensuring therapeutic effects. In recent years, the microvesicles – both their total pool and their separate fractions – have been paid close attention (the closest attention has been paid to the fractions, indicated as "exosomes"). Some researchers believe that it is the extracellular constituent — microvesicles — that has the main therapeutic effect, while the functions of MSCs are limited to the formation of vesicles. They are already being tested as an independent therapeutic means, and at least in the experiments with animals, they really demonstrate a very vivid therapeutic impact. All this has been described in detail and summarized in the reviews, dedicated to the MSCs microvesicles [14, 15].

In addition to the distant impact, there is also the contact effect of MSCs, which was phenomenologically described in rather fine detail. Yet, due to evident technological complexities, almost all the data about the contact effects of MSCs have been studied in the *in vitro* systems and extrapolated to the notion of what is happening *in vivo*. However, there are some limited data about the direct contact interactions between MSCs and the differentiated cells immediately in the organism, *in vivo*. It seems that the "targets" may be all the types of differentiated and non-differentiated cells. At least they were registered in all the cases where these interactions were studied. And the direct contacts between MSCs and target cells cover all the variants of this type of interactions. Small molecules and electric signals come via gap junctions. All types of molecules and cellular organelles go through the microtubules. During the dissolution and resorption of membranes in the contact area, local subunits of MSCs and their targets are formed. Partial fusion can lead to the replacement of the damaged content of the affected "target cell". And complete fusion can potentially replace the entire content and preserve and renew the differentiated cell.

Direct contact interactions between MSCs and damaged cells in the *in vitro* and *ex vivo* systems have been studied from many angles. Nevertheless, this very way of preserving and restoring the damaged specialized cells as a specific therapeutic field of MSCs action is practically paid no attention on the level of clinical trials. Due attention is not paid in the sense that they are not registered, not evaluated in terms of quantity and types of contacts. First of all, it is related to an extremely poor state of knowledge about this process *in vivo* and the absence of technological solutions, which would allow managing these interactions *in vivo*. At the same time, even scarce current experiments *in vivo* demonstrate exclusive possibilities of direct interactions. With all the assumptions, it is yet unclear how lifelong non-dividing and almost non-replaceable human cells work smoothly and very intensively throughout the entire "species duration" — 100 years. First of all, it is related to cardiomyocytes and neurons. And the replacement of the old, damaged content of the cell with the new content from MSCs [16], complete fusion of these cells with MSCs [17] is

likely capable of restoring them during the entire "species duration" and in the widest range of damages. The very fact of this fusion with neurons was experimentally reproduced in the experiments*o*. Moreover, it was demonstrated that it actually takes place at the norm, in a quiet state, and gets more intense at the effects, capable of enhancing the damage [18] (Fig. 4).

All kinds of stem cells (except for MSC) also belong to irreplaceable ones. They are self-recreating themselves and thus seem to exist in the organism throughout its "species duration". But it does not save from damageability (as stated above). They also need to get rid of the damages, irremovable on their own. And it is not "accidental" that stem cells are located in particular "niches" — special cellular environments. MSCs are always present in such niches too. And their connection to the stem cells in the niches is very consistent. Partial fusions, content transfer, and content exchange (removal of the damaged content and introduction of the new one instead) may provide stem cells with the "potential" for 100 years. And such direct contacts, fusions, may become the unique therapeutic means which is capable of supporting, preserving, restoring any tissues and organs, including the ones that are most problematic for treatment.

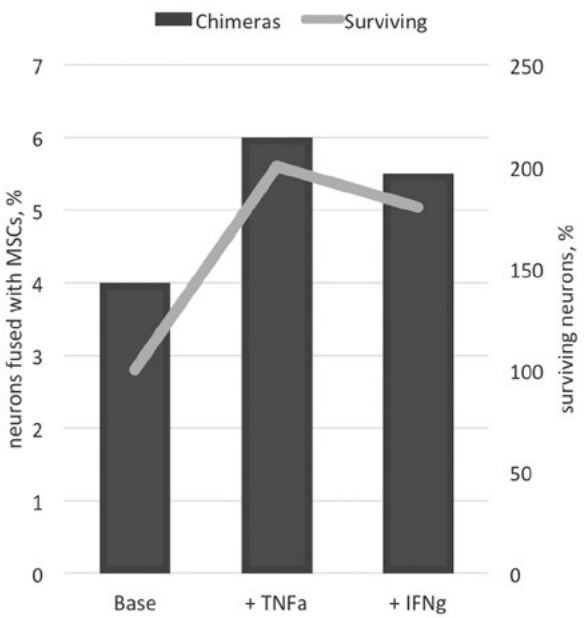

Fig. 4. Inflammatory factors TNFα and IFNγ enhance the fusion of MSCs with neurons and increase their survival *in vitro*. (Modify by Kemp *et al.*, [18])

As one of the direct contact variants, complete consumption of MSCs by the immune system cells is considered one of their main functions *in vivo*. According to these notions about the mechanism of therapeutic effect of MSC, this is a terminal form of contact interactions, a so-called "kamikaze effect". Thereby, MSCs induce their consumption by a macrophage and thus reform it to implement the tasks of repairing damages in the organism. Actually, only macrophages (monocytes — in the blood) are "true" therapeutic agents: "… infused MSCs are rapidly phagocytosed by monocytes, which subsequently migrate from the lungs to other body sites. Phagocytosis of ucMSC induces phenotypical and functional changes in monocytes, which subsequently modulate cells of the adaptive immune system. It can be concluded that monocytes play a crucial role in mediating, distributing, and transferring the immunomodulatory effect of MSC." [19].

Some scientists voice their ideas on some "total full-scale" effect of MSCs. This variant envisages that having penetrated the organism, the introduced MSCs implement everything described about them in the scientific litera-

ture, in turn. One of the variants of this integrally summarized set of properties was even ambitiously presented as a "MSC theorem" [20].

All these distant and contact mechanisms of potential therapeutic effect of MSCs are demonstrated very vividly, traced second by second, reproduced many times, and make a very strong effect. Yet, it is almost always done, shown, *etc.* in the systems *in vitro*. And then, it is extrapolated *in vivo*. A different noteworthy situation was found for exosomes. Their isolation, the study of their composition, the consumption by cells, and the changes in the properties of specific cells have been investigated (and is still being investigated) *in vitro* as well. But the integral effect on the organism is intensely studied *in vivo*. This situation is "two-fold". The exosomes are obtained in the *in vitro* system out of MSC, growing in the *in vitro* system, again, outside the organism. Therefore, the exosomes, obtained for the study of therapeutic effects are actually "*in vitro* twice" — the very MSCs are grown *in vitro*, and then, also in the *in vitro* system, these "*in vitro* MSCs" are used to obtain the *in vitro* exosomes. Under normal introduction of MSCs into the organism, they should (by the essence of their biological functions) get reformatted for the repair of the damage in the organism, organ, tissue, and differentiated cell, — not "generally", but that of a specific organ, its differentiated cells, somehow damaged by something yet absolutely specifically. Being in the culture, MSCs are "in the waiting mode" functionally. Recently, a special term, "passive MSCs", has been used in the literature for such "*in vitro* MSCs". And there will be neither reformatting of them nor, as a result, reformatting the exosomes *in vivo*. As for the therapeutic effect on the organism after the introduction of these "*in vitro* exosomes", it is evaluated by the results in the *in vivo* systems.

Leaving the maximalism aside, one can assume that all the MSCs properties, obtained in the *in vitro* systems, actually get implemented in the organism, but not only by merely one variant, and not as shown in the pictures — successively, one by one, until "everything" is exhausted. In the organism, MSCs abilities are implemented in different quantitative and qualitative combinations, required to solve the tasks of preserving and restoring at each damage, according to this damage, and as applied to the tissue, organ, where the damage has originated.

Under all the relativity of extrapolation of the data, mechanisms, effects, *etc.* obtained *in vitro* — "in a tube" — upon the things, occurring in a living organism, and a sick one, in which normal mechanisms are changed, there is one very significant position, allowing to assume the plausibility of this extrapolation at least in terms of quality. The response of cells and their ensemble — the organism — are programmed in the genome. And if the response is registered, there is some "ability" for it in the form of some programme. And, in principle, there are no reasons to state that the same response, should it be truly possible, cannot be implemented in the organism. Because there is a "possibility", a programme for it. And the field of entwinements of endogenous factors, signalling, enzymatic and non-enzymatic reactions and their metabolites, and products is utterly more far-reaching than for any, and always limited, set of conditions in

the experiment. To implement the MSCs functions in the organism, their range of possibilities should be unique in all respects, much broader and more diverse than "in the tube". How plausible it is in terms of existing "possibilities", i.e. implementation of the information, present in the genome, is vividly demonstrated by the spectrum and levels of reformatting MSCs while simulating the requirements of the organism to their functions "as per the task". The most vivid demonstration for it was done on polar positions — the changes in MSCs, occurring under the effect of simulating "polar" signals. On one pole, these signals and a response to it were the simulations of a threat, and on the opposite pole — the signals, simulating the need to implement the preserving-restoring function of MSCs.

These "polarized" MSCs were marked as MSC M1 and MSC M2, respectively. Then the letter M was omitted, and the standard marking became MSC1 and MSC2. The fundamental quantitative and qualitative changes in the composition of macromolecules in these states of cells are well demonstrated by the two-dimensional phoresis of proteins (Fig. 5).

It is evident that the changes have occurred even on the level of conservative macromolecules and involved the charge of the proteins on the cell, which is reflected in the value of their Pi [21]. And the difference in the composition, number, and properties of proteins of these two phoregrams is the visualized polarization as per the task — to extinguish the inflammation or to repair damages. And the numbers of a series of signalling molecules in polar states were different, sometimes, the difference amounted to several orders (Fig. 6).

All these data make up a gigantic bulk of information in the literature which demon-

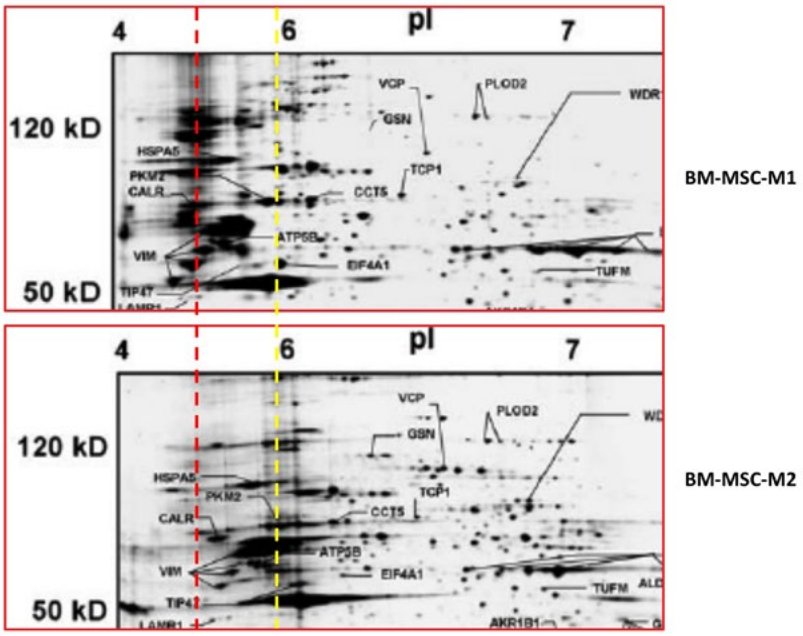

Fig. 5. Fragment of two-dimensional electrophoresis of MSC proteins in two polar states, M1 and M2. (Modified from [21]) MSC reformatting as per the task is accompanied with practically general cellular changes in proteins. Many minor proteins vanish in one state and appear in the other. The main structural proteins change their charge, which is evident in the change of their pI. It occurs during their modification — phosphorylation, acetylation, methylation, *etc.*

strates how everything may take place. Yet, it cannot be used to make up a picture of what the *in vivo* "actually" is. According to current notions, MSCs are new, highly efficient, *etc.* "therapeutic means". But if the main principal data, obtained *in vivo*, are united, i.e. under administration both to sick animals in the experiment and to sick people, where MSCs were applied for therapeutic reasons and in clinical trials, the resulting phenomenology is extremely unusual, though absolutely real, checked and confirmed multiple times, radically different from what takes place when usual therapeutic means are used. The first noteworthy thing is the temporal parameter of therapeutic effects. As a rule, MSCs are administered only once both in the experiments on animals, and in therapeutic procedures on people. Then, in case of severe chronic damage, the process of recovery begins. But the first signs of recovery or relief are observed not immediately but only a few days or weeks later. And then the process gradually, in the course of months, goes on until the recovery or some "healthier" level (Fig. 7).

This is known for the known medicines. As a rule, medicines should be taken either in long-term courses or lifelong. There are medicines of "immediate effect". These are pain relievers, nitro-glycerine, *etc.* But they do not treat anything, they just relieve or block this episode. By their principles of action, medicinal agents, applied for chronic or severe damage, can compensate for the weakened function, decrease its hyperactivity, replace (make up for) a quantitatively insufficient metabolite, a signalling molecule, *etc.* According to the mechanisms, which lie in their foundation, they cannot act in any other way. The exception is found in antibiotics (and the agents of the same mode of action). They remove (kill, destroy) the outer (bacteria, fungi, viruses) pathogenic agent or the inner one (tumours). The mode of their action is to suppress the agent, which has penetrated from the outside or occurred inside, causing the pathological manifestations, which allows the organism to eliminate this agent and come back to the norm. According to the current practice of

3 MSC donors in four independent experiments			
Protein	unprimed	MSC1	MSC2
IL2R	0	0	41.3
IL4	0.5	1.71	3.99
IL10	32.8	39.5	33.6
IL6	**414**	**7287**	**39987**
IL12p40	0	0	11.5
HGF	256	236	187.9
IL8	**45**	**6998**	**71233**
CCL10	**0**	**413.3**	**181777**
IFN	66.3	336.9	699.4
TNF	8.1	51.8	501.3
VEGF	2058	3213.7	2713

Fig. 6. The comparative data on MSC formation in different statuses of biologically active products. (Modified from Waterman R.S. *et al.* [22]and their activation leads to profound cellular and systemic responses that mobilize innate and adaptive host immune cells. The danger signals that trigger TLRs are released following most tissue pathologies. Since danger signals recruit immune cells to sites of injury, we reasoned that hMSCs might be recruited in a similar way. Indeed, we found that hMSCs express several TLRs (e.g., TLR3 and TLR4). Due to the metabolism reformatting, the number of biologically active macromolecules changes in terms of orders. When these quantitative data are compared against the values of the two-dimensional electrophoresis, they demonstrate the scale of the processes taking place in MSC when "the task is formulated" on the molecular level.

treating chronic damages, other principles of its treatment are applied. The medicinal agent should be taken either all the time or in courses. Yet the practice of application and the study of MSCs demonstrate that it occurs exactly in a way that "cannot happen". But this is true for chronic states. If there is an acute process, and MSCs are applied as a therapeutic means to eliminate it, the recovery comes very fast, in some cases — in a matter of several hours or even minutes (Fig. 8).

Yet, considering all these temporal specificities of the therapeutic effect of MSC, their distribution in the organism after the systemic (intravenous) administration is described almost everywhere in the same way, in princip-

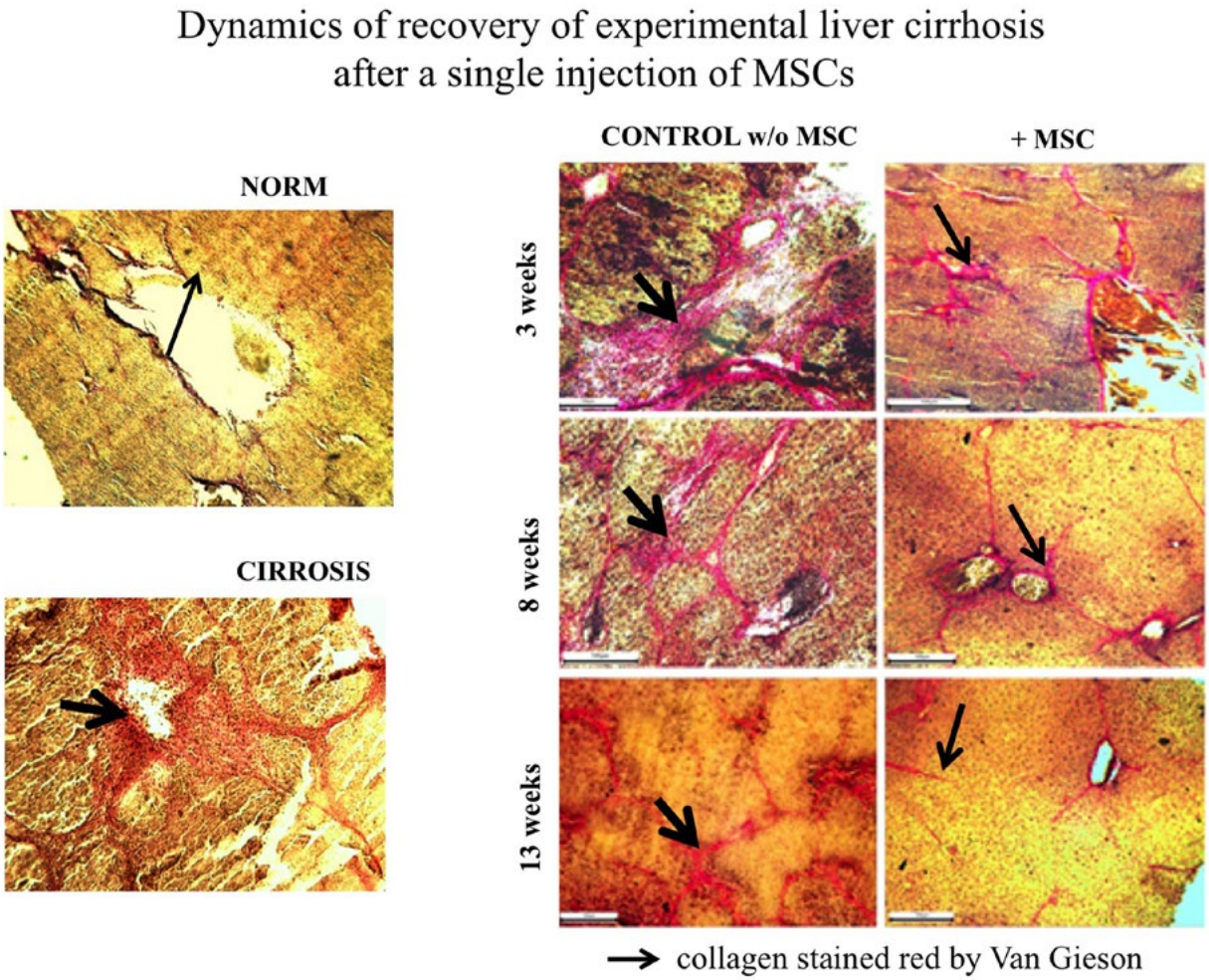

Fig. 7. The dynamics of liver restoration in rats with hepatic cirrhosis, induced by the administration of CCl_4. According to Rymar S. *et al.* [23]. Under a single administration of MSC, the process of restoring serious damages in the liver starts and develops for a long time.

le [19] after which they are trapped in the lungs and die and disappear within a day. The fate of MSC after their disappearance from the lungs is unknown and it is unclear how MSC realize their immunomodulatory effects in their short lifespan. We examined immunological mechanisms determining the fate of infused MSC and the immunomodulatory response associated with it. Tracking viable and dead human umbilical cord MSC (ucMSC. As early as five minutes after being injected, MSCs are mainly concentrated in the lungs and partially in the liver (in mice as model objects). And in the course of these five minutes in the organism, one third of the cells dies. In 24 h, their content in the organism (in the same lungs and liver) decreases more than twice and almost all of them are dead. Then, the number of MSCs drops rapidly. On the fourth day, only their trace amount is registered (Fig. 9).

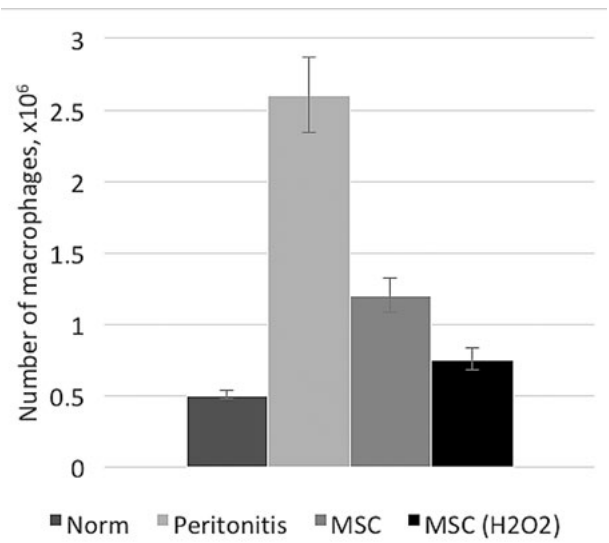

Fig. 8. The inhibition of the acute inflammation by the administration of low doses of native and previously conditioned MSC by H_2O_2 into the abdominal cavity of mice. The dose of native and preconditioned MSC — 5×10^3 cells/mouse. MSCs were administered 24h after the administration of peptone. The dynamics of acute inflammation after MSC induction was studied one hour later. In case of acute damage, the therapeutic effect starts very fast. According of Rymar *et al.* [23]

And in severe damages, the therapeutic effect of MSCs only starts manifesting itself several days or weeks later, under the best scenario (Fig. 10) [24].

As stated above, in recent years, the effect of MSCs is often related to their extracellular structured products — the extracellular vesicles.

Their detailed study demonstrated that both in their composition and therapeutic effects, they potentially, and in many cases actually, possess the properties of MSC. So, the notion has arisen that, as cells, MSCs are not required for the therapeutic effect at all, and their entire function comes to the production of microvesicles. At the same time, there have been some articles, demonstrating that no extracellular products whatsoever are required for the therapeutic effect of MSC. And the active full-fledged cells — MSCs — are not required either. On the contrary, efficient immunomodulation requires MSCs to be in the apoptosis state [25]. And the dead cells trigger the same therapeutic effect as the living ones, in the same terms, counted in hours: "Recently, we observed that inactivation of MSCs in which their immunophenotype remained intact while their secretome and active crosstalk with immune cells was disabled, retained the cells' immunomodulatory capacity in a lipopolysaccharide (LPS) sepsis model.

In this model, the therapeutic effect of MSCs appears to be independent of their cellular activity and depends on a mechanism

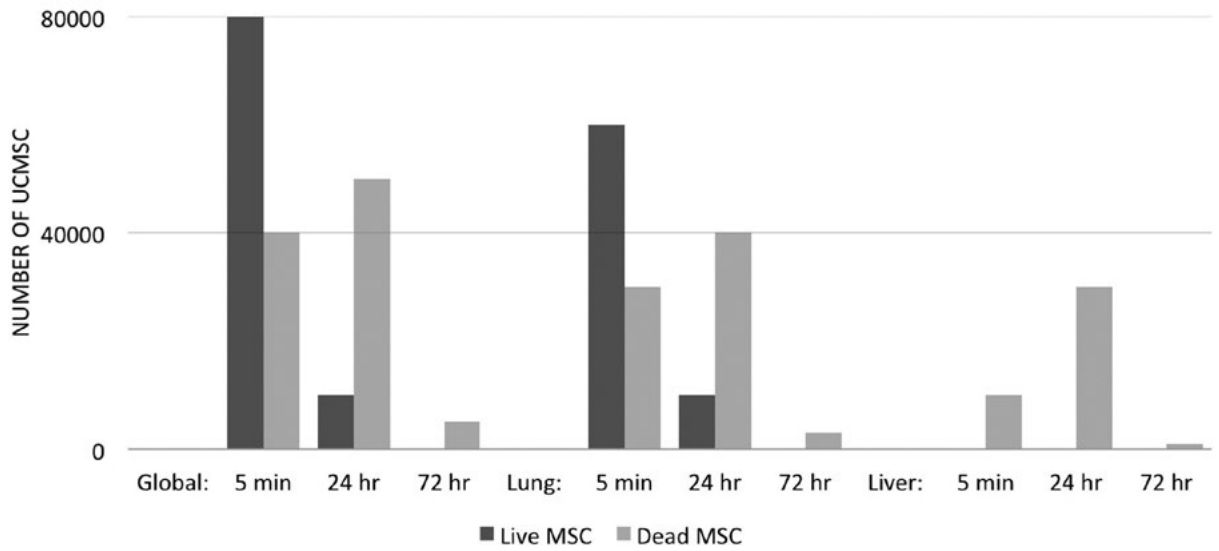

Fig. 9. The dynamics of MSC distribution after the intravenous administration. (Modified from De Witte SFH *et al.* [19]). After the systemic administration, MSC cells start dying immediately. Their distribution is mainly limited to the lungs and liver, from which they are eliminated quickly.

potentially involving recognition and phagocytosis of MSCs by monocytic cells." [19].

Sorting through these utterly contradicting results requires the quantitative (and possibly qualitative) evaluation of what is occurring in the organism. An organism is a strictly rational system. It has everything, in a minimally sufficient amount. And the direct determination of the MSCs amount in the human organism at the norm has demonstrated that it is miserably low. The highest determined concentration of MSCs is in the bone marrow — approximately 3.5 thousand of MSCs in 1 ml of the aspirate [26]. They are either found as single cells or not found at all [27]. Let us take the total amount of the bone marrow in a mouse as the volume of 0.1 ml and thus a proportional content of MSCs in them, as in humans. This gives us a value of ≈ 350 MSCs per mouse. From 1 to 10 MSCs in 1 ml are found in the blood of healthy mice. Let us round all this upwards and get the sum of about 500 cells. And this is 500 MSCs at the norm per one entire animal. The total number of MSCs for humans will correspond to their bodyweight. Let us take the volume of the entire bone marrow in humans as 100 ml. It will contain $\approx 3.6 \times 10^5$ MSCs (3.5×10^3 MSCs in 1 ml of bone marrow $\times$ 10^2 ml of bone marrow). MSCs are practically not found in the human blood at the norm, in a quiet state. There is nothing for them to do there. Something "working" is present in the tissues. Even if this amount matches that in the bone marrow, we have $\approx 7 \times 10^5$ cells ("items") per one entire human being. But this number of them corresponds to the needs of the time, when everything is "in order". It is in equilibrium i.e. there is constant disbursement for the implementation

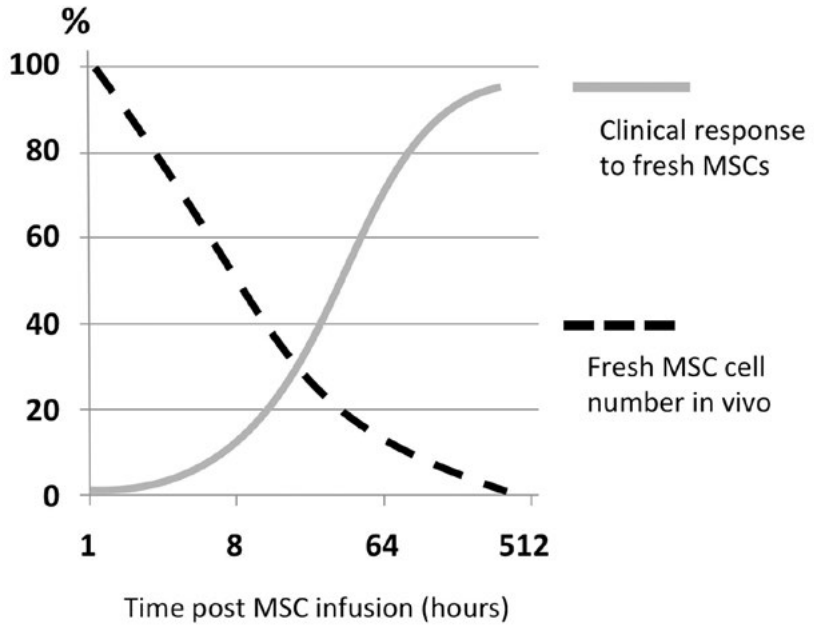

Fig. 10. Temporal specificities of MSC in the organism. (Modified from Moll G. *et al.*[24]. The presence of the administered MSC in the organism is utterly opposite timewise. MSC vanish quickly, yet their therapeutic effect starts when MSCs have almost vanished, and develops after MSCs have disappeared completely.

of MSCs functions and replacement of the "drop-outs". Usual stem cells can be replaced only "by themselves" — via self-reproduction. But MSCs division was not observed in the organism, it has not been described in the scientific literature. MSCs do not divide in the organism. They are formed in the organism. And not out of stem cells (there are none like this for MSC) but out of the differentiated ones. In the organism, MSCs are formed out of "progenitors" — usual differentiated cells "as required" i.e. when the organism needs them: "Thus, blood vessel walls harbor a reserve of progenitor cells that may be integral to the origin of the elusive MSCs and other related adult stem cells." [28].

Actually, there is nothing surprising in it. All the human cells (except for erythrocytes) carry the complete genome. And the cloned animals are a vivid demonstration of the fact that it can be implemented in the full scale. Pluripotent stem cells, according to Yamanaki, are formed due to the expression or introduction of only five signalling molecules, used to treat normal somatic cells of the adult organism (or to activate the corresponding genes) [29]. And MSCs are far from being pluripotent, and are formed not in the tube, where the selection and use of signalling molecules are limited, but in the organism. Their number and combinations therein are unlimited. And the cells, according to Yamanaki, are a miserable variant of almost endless possibilities of the organism. Therefore, there is nothing unusual in the MSCs formation out of the differentiated cells.

All this is coordinated and occurs constantly in the organism *in vivo*. And it is imple-

mented in a very rigid form in the laboratory dishware, in a "flask" (glass and plastic), in the "culture medium" where the jewel in the crown of the *in vivo* residues is fetal bovine serum. And according to modern tendencies, the culture media for MSCs do not even contain proteins. Yet, MSCs are extremely reformatively active, mobile, and highly reactive in their tasks in the organism. And they get reformatted under new conditions in the "flask".

A human cell is generally a self-sufficient system, and MSCs are extremely self-sufficient. In the organism, they implement only a part of the genome in its relevant site, "required" for the system, under the signalling-informational pressing of the system (the organism). On coming to a vacant space, free from the pressing of the controlling-managing systems of the organism, the cells start implementing their self-sufficiency, getting transformed into a form of life, capable of "everything", autonomously free but within the boundaries of its genome. And in the context of current technologies, this is the transformation into what we see and study as MSCs. In a figural expression, "MSCs are a phenomenon of the *in vitro* culture". [30]. In the organism, MSCs do not multiply; they are formed out of the cells of the internal endothelium of small and medium vessels — pericytes, endothelial vessels or vascular smooth muscle cells, *etc.*

To obtain the "material" for MSCs multiplication by placing it into the "flask", a "sample" is taken. A "sample" is a fragment of completely functionally and architecturally healthy tissue (bone marrow, adipose tissue, *etc.*) that is "taken" by mechanical destruction of the tissue. As a result, the cells in this "sample" receive the emergency signal of extreme danger in the very course of this procedure (mechanic destruction). The response to this signal should involve everything that accompanies a trauma. This includes the need for MSC.

Having received the signals of maximal danger, the damage, the progenitors start regrouping immediately, producing MSCs [31]. Contrary to the organism, in the culture medium MSCs divide (the implementation of self-sufficiency of the complete genome after the organism control is over), change with passages, getting adjusted to life on plastic, glass, in the "culture medium" (Fig. 11). And these multiplied MSCs from the "flask" are introduced into the organism.

Regardless of a small number, in the organism MSCs make miracles, ensuring those long years of life which are called "species duration". But in the culture, they have reformatted for autonomous existence. And the main question arises — how and why do they act as "therapeutic means" then?

To understand the phenomenology of the *in vivo* action of the multiplied MSCs, it is necessary to take the global view of the following: 1) the MSCs functions in the organism; 2) the alignment with the specific needs of the organism, to provide for which MSCs are actually formed in the organism; and 3) the possibilities, the potential of MSCs from the "flask", introduced into the organism from the outside.

The central position, defining all the subsequent events, is the fact that at the norm, the organism is self-sufficient not only due to the systems, self-reproducing it (the organism) on all the levels, but also due to the systems of protection, preservation, and restoration.

To fulfil their functions successfully, these systems are organized into self-maintaining cycles. And at the norm, the level of the self-maintaining activity of the functions is regulated according to the need for the level of this activity required to maintain the norm. As long as these systems, their self-maintenance and regulation of the required level are not breached, the organism is healthy, and those destructive processes which surround it from the outside and occur inside it, are removed, and the sustained damages are repaired. It goes on constantly during the entire human life. And the pathology starts revealing itself, developing, and becoming chronic, when "something" in the systems of protection, preservation, and restoration ceases working for some reason, or the "level regulator" gets disrupted, thus this level has become insufficient for the restoration and further maintenance of the "norm". If it is not a constantly acting violator of the systems of protection, preservation, and restoration (chronic intoxication, radioactive radiation, unceasing infection, *etc.*), then the system can be returned back to the norm. Yet, sometime later, the unceasing destructive impact causes the re-tuning of the level regulator, which re-tunes to a higher level of equilibrium, accepting it as a "norm". In this case, to achieve the restoration, the activity of the destruction factor should be decreased first, and the system should be returned to its initial

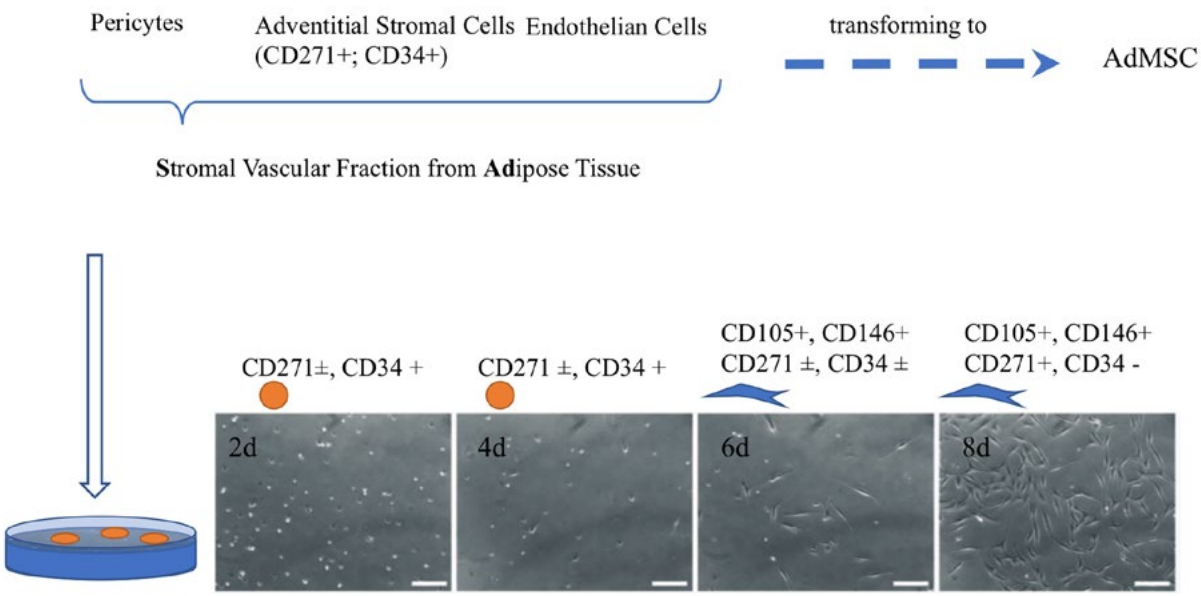

Fig. 11. In the early phase of *in vitro* cultivation of the stromal vascular fraction of adipose tissue cells (AdSC), adventitial cells are rearranged. The first 3 days there is a loss of cells of the original fraction and a "natural" functional selection of cells capable of attaching to plastic. On the 4th day, spindle cells appear, in which markers CD105, CD146 and CD271 appear. With the advent of CD105, CD34 expression is suppressed by the 8th day, and the cells acquire a typical MSC immunophenotype. (Modified from Braun J. *et al.*[31])

"normal" state. And this is the central point of the problem. A "normal" state is not only the activity level of the protection system; it is also self-supply and self-maintenance for this level, ensuring protection, preservation, and restoration.

There are a few MSCs in free form in the organism. Their formation requires some time. And it takes place in the natural framework of the needs for MSCs. And the self-maintenance of the equilibrium state of the destruction/restoration systems in the cells gets disrupted under the unceasing increase in the destructive processes. The restoration systems cannot repair the damages, occurring more than at the norm, fast enough, and the equilibrium state shifts towards the increase in the level of damages. One of these well-known and well-studied mechanisms, constantly breaching the equilibrium of the destruction/restoration system, is chronic inflammation. The inflammation (any) along a long chain (cascade) of already well-studied, signalling-metabolically-energetic processes comes to the terminal stage — the formation of the reactive products (ROS) — peroxides, radicals, reactive species of oxygen, nitrogen. A specific level of ROS is required at the norm. Its decrease below this level leads to pathological violations. At the norm, the formation of ROS is a normal, necessary function of the metabolism and energetics of the cell. At the norm, their level fluctuates within a certain range which ensures the equilibrium (also normal) state of the systems, ROS-forming and ROS-transferring into inactive forms. In case of acute damage to the organism, there is rapid activation of ROS along the cascades of the signalling-regulatory systems. It starts with the release of "alarm" molecules out of the cells of anti-inflammatory cytokines and other signalling molecules. Joining their receptors, they activate the ROS-forming systems. As the consequences of the acute breach are removed, the activity of the formation of pro-inflammatory cytokines decreases, and everything comes back to the norm. However, in chronic states, the level of pro-inflammatory cytokines and other pro-inflammatory signalling molecules is increased and, along the chain of the processes, the level of ROS stays high. The chronic activation of ROS is phenotypically implemented into what actually is "inflammation". At the norm, this is one of the mechanisms, protecting the organism from imminent and massive penetration of the alive, dangerous xenogeneic agents (bacteria, fungi, viruses, organic and inorganic toxic "harmful" products, *etc.*), surrounding the organism, inside the latter, or an organ, a tissue via the damage which has occurred. And if the norm — the preservation of the increased level of "ROS-signalling" — is breached, it is a pathology.

There is no "stability" for radicals, peroxides, *etc.* They oxidize everything they can oxidize energetically. This is how all the foreign items that have penetrated the damage from the outside are eliminated. But there are no "insiders" and "outsiders" for radicals and peroxides. Everything, including the insiders, is eliminated. The "insiders" are somewhat protected by the ROS-inactivation system, present in each cell. But this is for the norm. Although it is only partially true even for the norm. Non-deactivated ROS use their non-deactivated reactive molecules to destroy the content of the very cell. After the emergency activation of ROS, the systems of its control

bring them back to the norm; the repair systems remove, and repair the damages. The systems of the cellular level restore the macrodamages and everything comes back to the norm. But in chronic states, due to the occurring self-maintaining cycles, there is an increased level of the first link in the chronic chain — pro-inflammatory signalling molecules, leading to excessing the norm, the ROS level. It is not compensated by the level of the deactivation systems. The equilibrium is breached, the ROS level constantly becomes somewhat higher as compared to the norm. There is more intense damageability of macromolecules and cell structures. The reparation systems cannot cope with their restoration. The equilibrium in the synthesis/elimination of the damaged macromolecules is shifted. With time, being a new level, it is accepted as a "norm" and is self-maintained on this level. The equilibrium state shifts to a higher level of damageability due to the higher level of ROS formation. Their higher-level leads to the impairment of metabolism via the destruction of proteins of metabolism chains. The chronic state emerges. And then, as a result of this impairment to the self-maintenance level of the system of protecting and restoring to the level, insufficient for the norm, it becomes a self-maintaining pathological state of the second pathological "norm". As "everything" is a target for ROS, the decrease in the amount and activity of proteins and structures, carrying out antioxidant, repairing, "trash"-removing processes, *etc.*, occurs as a part of this "everything". As a result, it leads to the increased damageability of the organism cells, of the entire organism, or just one tissue or organ. The capacity of the MSCs, which are formed by the signal for the need in this state, and are also present in the conditions, which led to the insufficient level of the entire system, is lacking to bring the systems of protection and restoration back to the "norm". Gradually all this destroys the organism. The chronic state develops. And this is the background of the organism state for implementing the potential of the introduced MSC. There are some data, presented above to demonstrate that there are only ≈ 500 MSCs in the organism of a mouse at the norm. And the mouse is usually administered 100,000 (or even one million) MSCs simultaneously. It is ≈ 200 times more than it usually contains in the equilibrium state. The same ratio is true for humans. The highest dose for systemic (intravenous) administration of MSCs to humans, mentioned in the scientific literature, is usually 100–200 million ($1–2\times10^8$). And according to the calculations above, at the norm, humans have $\approx 7 \times 10^5$ MSCs, i.e. ≈ 150–300 times less. Under the local administration (for example, in orthopaedics), the administered dose is lower — about 10–50 thousand MSCs. But locally, there are not too many resident MSCs in proportion to the tissue volume, where the "dose" has been administered. Massively administered from the outside, fresh, MSCs immediately start performing the task, for which they exist in the organism — the normalization of all the systems of protection, preservation, and restoration. But normalization is also the normalization of the self-maintenance level. It requires removing the pressing which has brought it to the abnormal level. It is the inflammation, the consequence of the sustained damages, impairments, *etc.* The self-maintaining chain, increa-

sing the activity of "ROS-signalling" should be broken. To remove all this, MSCs have the entire spectrum of actions and mechanisms of implementing them. The breakage of the obligatory increase in ROS achieves the main thing — bringing the systems of preservation and restoration to the appropriate level, their self-maintenance, ensuring this (appropriate) level. And then, these very systems of restoration, brought to the appropriate level, start their self-treatment. MSCs do not divide in the organism, they are formed out of the differentiated cells, which have to perform their specialized functions as well. Their potential to form MSCs is not endless. After being administered from the outside in a large amount (as compared to the resident ones), MSCs immediately start performing the functions of protection and restoration on a very high level of their preserving-restoring mechanisms. These are the elimination of the factors, causing the inhibition of the resident systems of preservation-restoration and bringing them to the appropriate level of self-maintenance and the fixation of this level. The level of "Self...". Then (and only then), after the administered MSCs have restored the self-maintenance of the resident preservation and restoration systems, required to repair the damages, these systems will be able to ensure the self-repair of breaches, damages, deviations, *etc.* in a sick organism just like at the "norm". Even being insufficiently active in a sick organism, all these systems are still present therein. They do not have to be formed anew: transferring heterochromatin into euchromatin, switching on new signalling chains, ensuring the activation of the relevant genes, *etc.* All this is already both present and working there. But not at the appropriate level. And it cannot bring itself to the appropriate level and self-maintain it. The task of MSCs is to ensure this level. To achieve this task, during its restoration and setting the level regulator "right", they should remove, block whatever does not allow the entire system to overcome the pressure of negative factors of the state of the cell, the organism (inflammation, excessive products of peroxides and radicals, impaired coordination between metabolic cycles and chains of signalling, *etc.*). This is the crux, essence, and specificity of MSCs functions in the organism. In the organism, MSCs are formed not for their own existence but to ensure the existence of other cells. In the organism, MSCs do not have the functions of self-maintenance. In the organism, MSCs have only the function of using themselves to restore other cells. This is their potential — to be reformatted according to the needs of the damaged, worn-out, weakened specialized cells and to use themselves, their entire pool of proteins, nucleic acids, structures, *etc.* to save, to bring their own systems of protection and restoration to the level, required for their "normal" functioning. On entering the culture, the fundamental ability of the mammalian cells comes into action in MSCs — self-sufficiency. The reformatting of MSCs starts going in this direction now. MSCs become "themselves for themselves". And a few divisions later, only a basic level is left. The basic "tissue-specific" level, this property of all the primary cultures of cells. Being the residual one, it is present in all the initial cultures of specialized cells for some time. For MSCs, their "tissue-specific" level is the protecting-defending-restoring potential. Under all the different variants

of MSCs administration into the organism, this residual potential (of distant and contact effect) implements those few phenotypic therapeutic manifestations, registered in the experiments. This is the residue of that basic "tissue-specific" constituent of MSCs, which is preserved under any reformatting. In the organism, it is required for the first immediate reaction at the specifics of danger, not identified by the intercellular, intraorganism signalling yet. This is the universal response. This is the tissue-specific residue while cultivating MSCs "in the tube". It is not as efficient after the multiplication *in vitro* as the one, reformatted for the formed need, but at least it is universal — enhancing all the functions of preservation and restoration. And no matter in which form or state MSCs are used, this is what they are working with — their residual basic potential. As MSCs are administered in large amounts, their residual potential is sufficient. This is registered as a controversy which MSCs do not have by their nature. But all this is true for the application of the multiplied cellular mass, "worked out" outside of the organism.

Yet, in the organism, MSCs are the central instrument of the organism to self-maintain it "at the norm". MSCs are principally different from all the stem cells. One of the main functions of all the stem cells is self-preservation for self-recreation and then functioning as stem cells. MSCs have the only function in the organism; it is self-application of themselves to protect, preserve, and restore all the other cells in the organism, including stem cells and the "cells of the species duration". MSCs should use themselves maximally for it. All of them and completely. And, if MSCs are not defined by origin (and Caplan has already suggested this variant), then MSCs could be named by their main intended purpose in the organism — "mesenchymal preserving-restoring cells" — MPRC. And to achieve the maximal application of MSCs as a universal and highly efficient therapeutic means, we should find ways of "improving", perfecting, and transforming them while working with them "*in vitro*". And the first and basic task would be to learn how to multiply MSCs in such a way that in the "outcome", they would preserve their potential inherent to them *in vivo*. And then, due to the unique possibilities of reformatting, their therapeutic potential can be brought to any desired level. It is unreal to achieve it in the organism itself — any "required" effect on MSCs will lead to some side effects. There is an endless diversity of cells of different types, properties, states, *etc.* in the organism. Any effect "for/on MSC" in it will be harmful to something else. And there are only MSCs in the "flask". There are no side effects for the "flask". So MSCs can be prepared or transformed at will. And this is the future of cellular therapy which, based on the study results, has started its way of being worked out.

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MSC — що це?

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Інтерес до МСК стрімко зростає через їхні можливі та реальні терапевтичні властивості.

З точки зору наукових досліджень і клінічного застосування основна увага приділяється питанням, пов'язаним з використанням МСК як лікувального засобу. Успішне просування в цьому напрямку вимагає розуміння місця МСК в організмі та тих факторів, які зумовили необхідність еволюційного прояву функцій, які виконують спеціальні клітини – МСК.

Розглянуто сучасні уявлення про механізми терапевтичної дії МСК. Ці уявлення порівнюються з експериментальними даними про феноменологію терапевтичних ефектів, зареєстрованих після введення в організм МСК як лікувального засобу. Проаналізовано інформацію про особливу роль МСК в організмі та сформовано уявлення про сутність цієї особливої ролі. На основі проведеного аналізу висувається пропозиція щодо спрямування досліджень на посилення терапевтичних ефектів МСК як потенційно високоефективного лікарського засобу.

Ключові слова: МСК

Received 01.04.2022