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SWISS STEM CELL TECHNOLOGY

Evaluation of the possible influence of the Enerchip Vitacell Dog and Vitacell Cat of the company 4pets in Romanshorn (Switzerland) on the growth and the viability of adult mesenchymal stem cells from the fatty tissue of dogs and cats in vitro

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Introduction and objectives

The company 4pets from 8590 Romanshorn distributes a series of so-called enerchips, which are manufactured by the Swiss company Penergetic International AG. The enerchip Vitacell Dog for dogs and Vitacell Cat for cats are, according to the manufacturer's specifications, alleged to have health-promoting properties, which have above all a positive effect on the stem cells of the treated animals, and thus to increase the well-being and the health of the animal. Enerchips Vitacell are mineral-based chips that can be sticked into the bowl and are embossed by means of a biotechnological method, which transfers the effect via biomagnetic fields (scientific spins) in the order of Pico-Tesla 10^{-12} to the water.
Previous studies were carried out in terms of energetic science or based on declarations by animal owners.

Mesenchymal stem cells are adult stem cells, which can be found in all body tissues as reserve cells and are responsible for the replacement of used cells in the tissue. While, in the past, above all the stem cells from the umbilical cord and from the bone marrow were known, the fatty tissue is nowadays the ideal tissue for finding and isolating the stem cell. They can then be used for treatments in domains of the regenerative medicine. For instance, stem cells are used for arthrosis, tendon lesions, spinal marrow lesions and apoplexy for the animals and the humans. In addition, stem cells can be used to test negative and promoting effects on cell cultures also under laboratory conditions. These toxicity tests are generally used for testing new drug substances and reduce the use of animal experiments.

The objective of the study was to determine whether the Enerchips Vitacell Dog or Vitacell Cat would show an influence in the cell culture with stem cells of cats and dogs. As a sign of influence, the growth and vitality of the cells in the culture were tested either with or without the addition of an Enerchip in the first series of tests.

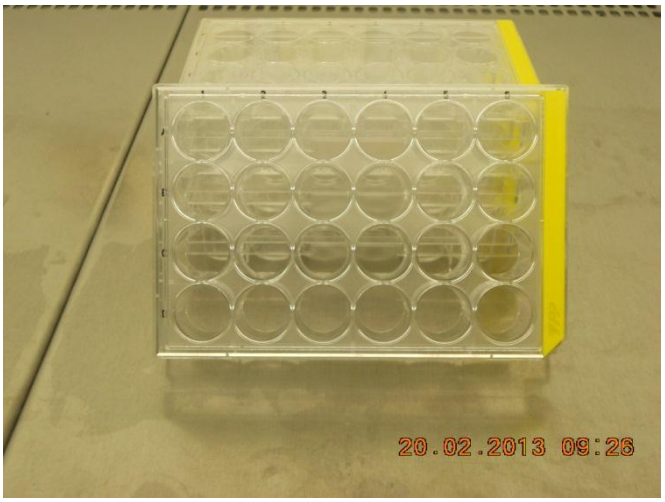
Material and methods

For the first test arrangement, adult stem cells of 10 different dogs and 10 different cats were used. All cells were originating from donors for which a hazelnut-sized piece of fatty tissue of the subcutis or of the stomach area were removed under normal chirurgical conditions. All dogs received in this process at the veterinarian's an anaesthesia by means of Detomidin, Morphasol and Ketamine hydrochloride, followed by an inhalation anaesthesia with Isofluran. The cats were narcotized by means of intramuscular administration of Detomidin and ketamine hydrochloride. For all animals, the primary objective was castration. The patients were of different races, genders and ages. The extracted fatty tissue was further processed accordingly at Vet Cell Europe in Muenchwilen. After the isolation of the stem cells, the latter were stored in liquid nitrogen. The isolation procedures, the storage of the cells and the de-icing process were the same for all animals. Around 500'000 cells were thawed for all animals. The thawed cells were grown in nutrient solution in an incubator at 37 ^{0}C and 5% CO_2. To avoid a widely diffused influence of the Enerchips, 2 separate incubators were used. To have a direct comparison, 2 stem cell doses were thawed per animal. One was fitted with the chip on the bottom of the bottle and the other without Enerchip was transferred into an identical culture flask. The number of cells per dose amounted to approximately 500'000 cells. The cells were observed over a period of 48 hours on a regular basis with the inverted microscope and photographically recorded. After 48 hours, the cell count was measured and compared with one another within the respective animal. The cells were measured with an automated cell counting device from the company Biorad.

In the second test arrangement, in turn, adult stem cells of 10 cats and 10 dogs were used. 2 series of mini well plates were loaded from each animal with a cell count of initially about 25 000 cells per well (see Picture 1). For each animal, 2 plates were filled with 10 wells (recesses) each and, in turn, one of the plates was fitted with the Enerchip Vitacell Dog or Vitacell Dog. The stem cells were distributed to the 12 wells and fitted with 0.5 ml of culture medium. During this test, the intracellular adenylate kinase was tested. Adenylate kinase is an enzyme which occurs only in the interior of the cell. Leakage of this enzyme indicates a cell damage. The damage can vary between a slightly elevated porosity of the cell and up to the death of the cell. Adenylate kinase can be used to drive a chemical process, which causes a substance to light up. Then, this luminosity can be measured in a luminometer. The measured value is referred to as relative light unit RLU. The higher this measured value, the higher the amount of adenylate kinase and the higher the damage to the cell.

The relative light units of all 12 wells were measured and compared with the corresponding animal once with and once without Enerchip.

Picture 1: Micro well plates



Results

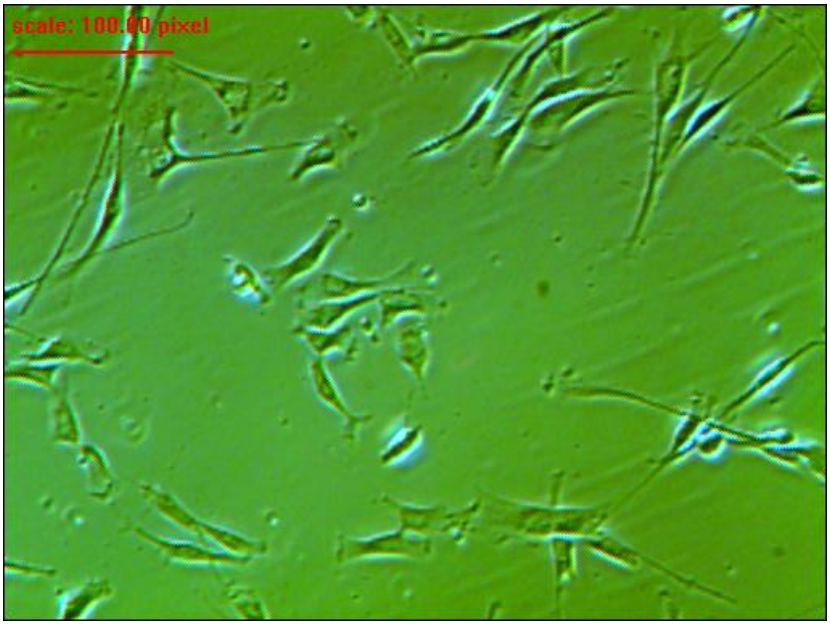
In the first test arrangement, the cells were unbound from the culture dish after 48 hours and counted. The average cell count for the dogs without Enerchip after 48 hours was 4.902 x 105 cells per milliliter, while the cell count for the dogs with addition of Enerchip Vitacell Dog was 6.259 x 105 cells per ml. This corresponds to an increase of 26.2 percent in the group, in which the Enerchip was used. The cell count for the dogs without chip varied from 2.29 x 10^5 to 8.2 x 10^5 cells per ml. The cell count in the dog group with Enerchip Vitacell Dog varied from 2.46 x 10^5 to 11.43 x 10^5 cells per ml. In no case, a reduced growth of cells through the influence of the Enerchips was observed. An increase in the proliferation of stem cells in the order between 4.08 and 50.04 percent was observed in the group with the Enerchip.

Table 1: Cell count for the dog with or without Enerchip and percentage increase in growth.

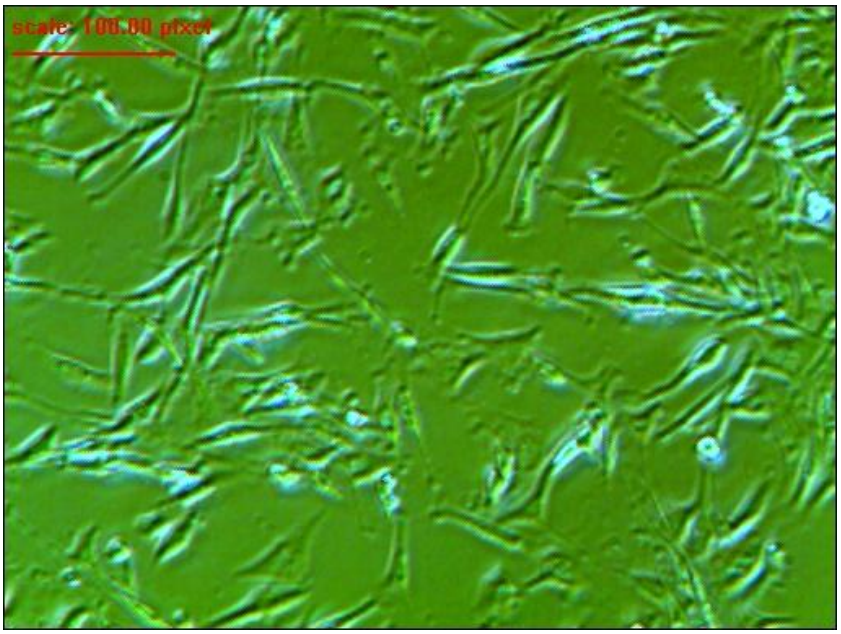
Dog no.	Cell count without chip	Cell count with chip	Delta %
1	3.83	5.76	50.04
2	8.2	11.43	39.39
3	6.71	9.72	44.86
4	6.96	8.4	20.69
5	2.29	2.46	7.42
6	3.38	4.83	42.9
7	7.11	7.4	4.08
8	2.44	2.63	7.79
9	4.97	6.01	20.93
10	3.13	3.95	26.2
Average	4,902	6,259	26.43

Cell count in 10^5 cells per milliliter and increase in percent

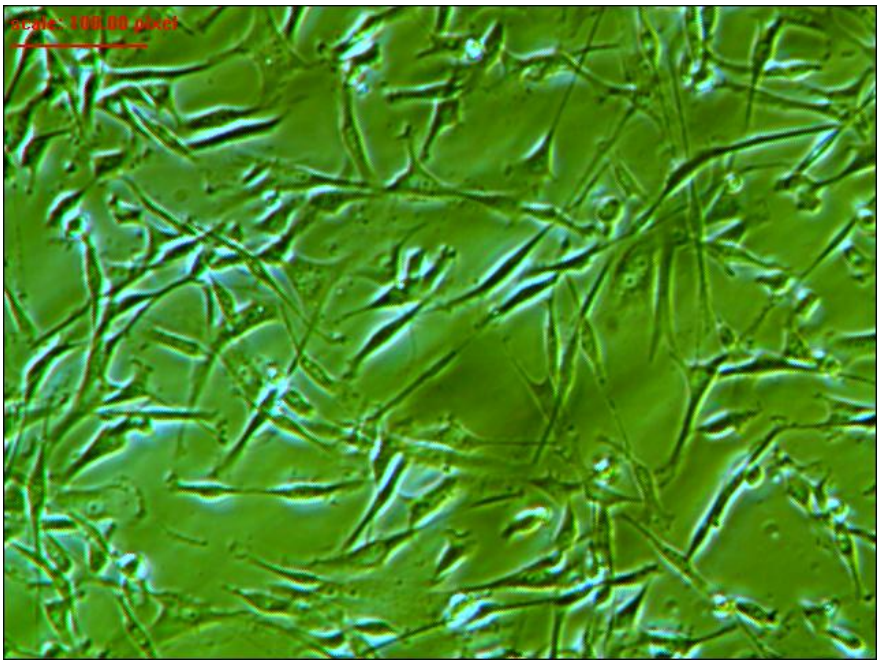
Picture 1: Dog stem cells for Dog no. 1 in growth WITHOUT Enerchip Vitacell Dog



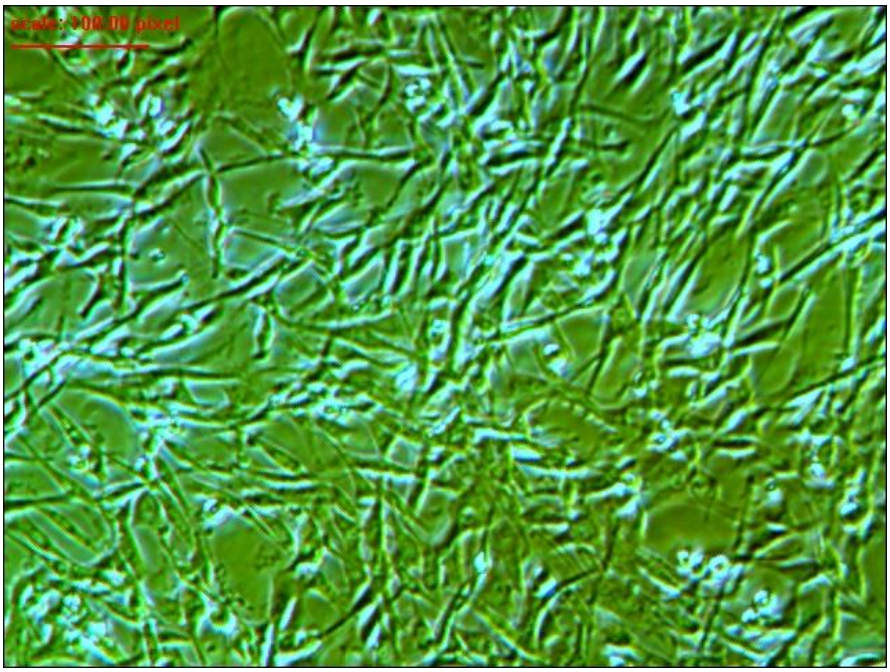
Picture 2: Dog stem cells for Dog no. 1 in growth WITH Enerchip Vitacell Dog



Picture 3: Dog stem cells for Dog no. 3, 48 hours of growth WITHOUT chip



Picture 4: Dog stem cells for Dog no. 3, 48 hours of growth WITH chip



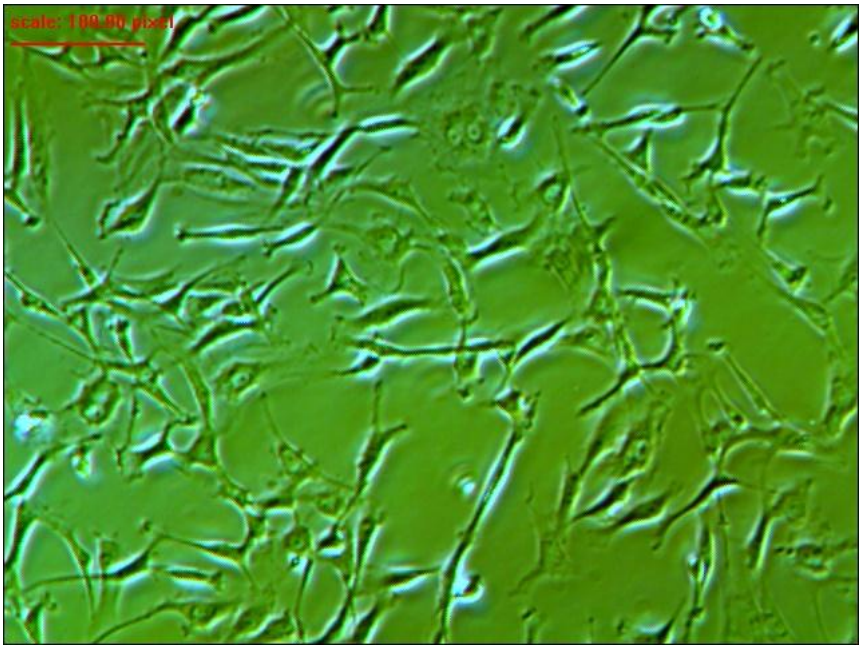
The average cell count for the cats without Enerchip after 48 hours was 1.68 x 10^5 cells per milliliter, while the cell count for the cats with addition of Enerchip Vitacell Cat was 2.11 x 10^5 cells per ml. This corresponds to an increase of 35.92 percent in the group, in which the Enerchip was used. The cell count for the cats without chip varied from 0.8 x 10^5 to 3.38 x 10^5 cells per ml. The cell count in the cat group with Enerchip Vitacell Cat varied from 1.31 x 10^5 to 4.2 x 10^5 cells per ml. In no case, a reduced growth of cells through the influence of the Enerchips was observed. An increase in the proliferation of stem cells in the order between 0.82 and 98.75 percent was observed in the group with the Enerchip.

Table 2: Cell count for the cat with or without Enerchip and percentage increase of in growth.

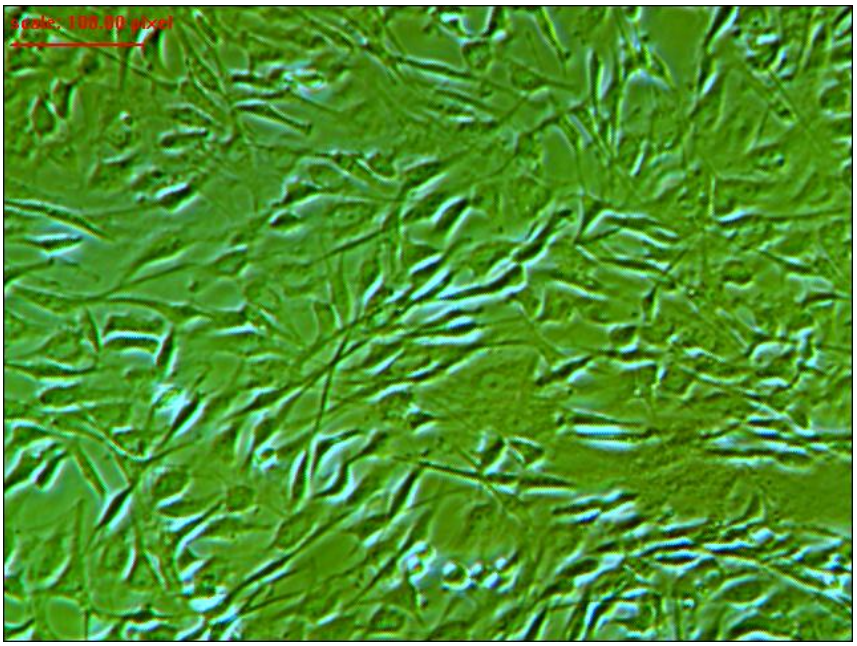
Cat	Cell count without chip	Cell count with chip	Delta
1	1.49	1.58	6
2	2.83	3.02	6.71
3	1.64	1.83	11.59
4	3.38	4.2	24.26
5	1.19	1.79	50.04
6	1.19	1.83	53.78
7	0.85	1.31	54.12
8	0.96	1.47	53.13
9	2.44	2.46	0.82
10	0.8	1.59	98.75
Average	1.68	2.11	35.92

Cell count in 10^5 cells per milliliter and increase in percent

Picture 5: Cat stem cells for Cat no. 7, 48 hours of growth WITHOUT a chip



Picture 5: Cat stem cells for Cat no. 7, 48 hours of growth WITHOUT a chip



In the second test arrangement, the amount of extracellular adenylate kinase was measured using the ToxiLight test. 2 times 10 wells were created for each patient, while a culture system was incubated without and one with Enerchip. The results of the 10 wells per dog and cat are listed in Tables 3 and 4. The average relative light values RLU in the wells with the Enerchip influences were 4759 RLU for the dog, while the systems without chip showed increased values of average 6552.2 RLU. On average, the values for the dogs without Enerchip were increased by 38.89 % compared with the values with Enerchip.

The average relative light values RLU in the wells with the Enerchip influences were 5450.48 RLU for the cat, while the systems without chip showed increased values of average 6395.49 RLU. On average, the values for the cats without Enerchip were increased by 17.72% compared with the values with Enerchip.

Table 3: Average values for cell damage in the dog

Dogs		with chip	without chip	Difference %
Dog no.	1	4433.2	6140.2	38.5
	2	5393.2	6990.8	29.62
	3	5595.7	6975.2	24.65
	4	5320.3	6059.7	13.9
	5	4393.5	6901.4	57.08
	6	4834.5	6952.2	43.8
	7	4744.7	5931.7	25.02
	8	4139.2	6474.3	56.41
	9	4374.1	6178.8	41.26
	10	4361.6	6918.1	58.61
	Average	4759	6552.2	38.89

Values in relative light units RLU

Table 4: Average values for cell damage in the cat

Cats		with chip	without chip	Difference %
Cat no.	1	5587.4	6446	15.37
	2	6758.4	7360.4	8.9
	3	7312.2	8516.6	16.47
	4	4642.7	5305.1	14.27
	5	5071	5866.7	15.68
	6	4955.8	6148	24.06
	7	5117.2	6215.7	21.47
	8	4894.9	6099.5	24.61
	9	5235.7	5917.6	13.02
	10	4929.5	6079.3	23.32
	Average	5450.48	6395.49	17.72

Values in relative light units RLU

Discussion

The company 4pets distributes a series of so-called Enerchips, which are manufactured by the Swiss company Penergetic International AG. The enerchip Vitacell Dog for dogs and Vitacell Cat for cats are, according to the manufacturer's specifications, alleged to guarantee energy exchange and thus to increase the health of the animals treated. The Enerchips are mineral-based energized chips and are enriched with a homeopathy-related method. The effect of the Enerchips is to be achieved via the positive influence of the drinking water or of the feed. Previous studies were carried out in terms of energetic science or based on declarations by animal owners.

Functioning of the action mechanism was not an object of the present study. The objective of the study was to determine whether the Enerchips Vitacell Dog or Vitacell Cat would show an influence in the cell culture with stem cells of cats and dogs. As a sign of influence, the growth and vitality of the cells in the culture were tested either with or without the addition of an Enerchip in the first series of tests.

Toxicological tests in laboratory on cell cultures have been carried out for quite some time and usually measure the permeability of the cell membrane by means of measurement of substances out of the cell, which do not reach outside under normal conditions. High doses of toxic substances cause a die-off of cells after the increased permeability of the cell membrane. Recent toxicological studies focus on adult mesenchymal stem cells from a fatty tissue. These stem cells are used not only like for a normal cell culture for toxicological studies, but can also give information on the possible damaging potential against the reserve cells of the body in the research on new medicines. Stem cells are relatively resistant to many drugs and chemical substances, so that, besides the toxicological tests, the growth behavior in the culture is also frequently is resorted on. The stem cell exhaustion syndrome known in humans, i.e. use up of the body stem cell reserves, has not been established in detail in the animal. On the other hand, most researchers equate the end of life with the end of the stem cell reserves. Under this approach, factors that positively or negatively affect the stem cells are of particular significance.

For the present series of tests, the growth behaviour of feline and canine stem cells from fatty tissue with or without the influence of the Enerchips Vitacell Dog and Vitacell Cat, respectively, was investigated. In addition, the vitality of the cells was tested. The simpler vitality tests, such as the trypan blue staining, are performed after removing the cells from the test, which are then no longer

available for further tests. In addition, this vitality staining of the stem cells are very inaccurate. Therefore, we have decided for the testing of the adenylate kinase. Adenylate kinase usually occurs in the interior of the cell and does not seep or only seeps in small amounts out of the cell. In our tests, the adenylate kinase drives the conversion of supplied adenosine diphosphate ADP into ATP adenosine triphosphate, in accordance with the formula Mg2 + ADP + ADP ----------Mg2 + ATP + AMP. Won ATP reacts with Luciferin via bioluminescence. Luciferin was found first in fireflies and also has a luminosity, which can be measured in a luminometer. The transformation is done from Luciferin to Oxyluciferin and light: ATP+ Luciferin + O2 ----------Oxyluciferin + AMP + PPi + CO2 + LIGHT. High light levels therefore indicate a collapse of the plasma membrane of the cell and thus a leakage of substances into the nutrient medium.

When applying about 500'000 cells to a culture dish of 75 cm^2, a growth to 3-5 millions of cells is to be expected within 2 days. Different growth behaviors are to be expected depending on the individual, so that variations are to be expected in a group.

For the dog stem cells, which were cultivated without Enerchip, an average cell count of 4.902 x 105 cells per ml of extracted cell suspension was measured. This corresponds to the usual experimental values in cell cultures of canine stems. The cell count for the dogs without chip varied from 2.29 x 10^5 to 8.2 x 10^5 cells per ml. This is also a number, which corresponds to the empirical values. The dogs were selected among a wide range of breeds, ages and initial quantities of fatty tissue.

The average cell count for the dogs with addition of Enerchip Vitacell Dog was 6.259 x 10^5 cells per ml. The cell count in the dog group with Enerchip Vitacell Dog varied from 2.46 x 10^5 to 11.43 x 10^5 cells per ml. This corresponds to an average growth of 26.2 percent in the group, in which the Enerchip was used. An increase in the proliferation of stem cells in the order between 4.08 and 50.04 percent was observed in the group with the Enerchip. In no case, a reduced growth of cells through the influence of the Enerchips was observed.

The average cell count for the cats without Enerchip after 48 hours was 1.68 x 10^5 cells per milliliter, while the average cell count for the cats with addition of Enerchip Vitacell Cat was 2.11 x 10^5 cells per ml. This corresponds to an increase of 35.92 percent in the group, in which the Enerchip was used. The cell count for the cats without chip varied from 0.8 x 10^5 to 3.38 x 10^5 cells per ml. The cell count in the cat group with Enerchip Vitacell Cat varied from 1.31 x 10^5

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Due to the sometimes extreme growth increase of up to 50% for the dog and up to 98% for the cat, the tests were repeated for the dog and cat stem cells concerned, with similar results.

The increase in the stem cell proliferation due to the influence of the Enerchip was extremely clear and very pronounced. Morphologically, no change was detected in the stem cells. Their behavior in the culture was normal in addition to the rapid pace of proliferation. In vivo, an increased proliferation of the stem cells would be advantageous for diseases with severe loss of tissue, for example heavy wear and tear of cartilage in the case of articulation problems, large cell death in the case of viral infections in the gastrointestinal tract or on respiratory mucous membranes for the cat cold complex. It is unclear whether the increased proliferation in vitro could be compared with a longer life in vivo. The stem cell exhaustion syndrome in humans assumes that stem cells in the age are no longer capable of proliferate equally well or quickly. In this case, a positive influence on the proliferation of stem cells would be of great significance.

With the second test arrangement, the vitality of stem cells in the culture was examined. It is known that some basic activity with regard to proliferation of the cells but also with regard to the artificial living conditions in the laboratory resulted in substances getting out of the cell interior. We have targetedly used growing cultures in our series of tests, which have a greater activity and therefore are more similar to the metabolism in vivo. To make a scientific statement, we have filled 2 x 10 wells from each stem cell donor with stem cells in order to make a more detailed statement within a donor. In turn, 10 wells of a patient were affected by addition of the Enerchip and 10 wells of a patient were incubated without any Enerchip.

The average relative light values RLU in the wells with the Enerchip influences were 4759 RLU for the dog, while the systems without chip showed increased values of average 6552.2 RLU. On average, the values for the dogs without Enerchip were increased by 38.89 % compared with the values with Enerchip.
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without Enerchip were increased by 17.72% compared with the values with Enerchip.

High RLU values indicate a pathological leakage of adenylate kinase from the cell interior into the nutrient medium of the cells. This suggests, for small amounts, friability of the plasma membrane of the cell. For high values, it is, however, a sign of the death of the cell. In our test arrangement, no cells have died in this process. The difference in the extent of the cell damages was, however, very pronounced and significant. Without the influence of the Enerchip Vitacell Dog, a boost of the RLU values and thus of the cell damage by 38.89 % was measured for the dog stem cells. Without the influence of the Enerchip Vitacell Cat on the cat stem cells, an increase of the RLU values by 17.72 % would be measured.

These values show the protection mechanism of the Enerchip on the viability and survivability of the stem cell. A longer life and an improved regenerative ability of the body can be considered for possible conclusions on the stem cells in the living body. In regenerative medicine, stem cells are used for replacement of used cells. The stem cells have the ability to convert into multiple cell types such as heart muscle cells, cartilage cells, bone cells tendon cells and nerve cells. The reason for this replacement from the outside is the small number of local stem cells or the multiplied local stem cells, wherein the existing cells were no longer able to carry out their duties. Should the stem cells in vivo also be influenced by the Enerchip, a fundamental increase for regeneration and self-healing is to be expected.

Abstract

The company 4pets distributes a series of so-called Enerchips. The enerchip Vitacell Dog for dogs and Vitacell Cat for cats are, according to the manufacturer's specifications, alleged to have health-promoting properties, which have above all a positive effect on the stem cells of the treated animals, and thus to increase the well-being and the health of the animal. Enerchips Vitacell are mineral-based chips that can be sticked into the bowl and are embossed by means of a biotechnological method, which transfers the effect via biomagnetic fields (scientific spins) in the order of Pico-Tesla 10^{-12} to the water. Previous studies were carried out in terms of energetic science or based on declarations by animal owners.

The objective of the study was to determine whether the Enerchips Vitacell Dog or Vitacell Cat would show an influence in the cell culture with stem cells of cats and dogs. As a sign of influence, the growth and vitality of the cells in the culture were tested either with or without the addition of an Enerchip in the first series of tests.

For the first test arrangement, adult stem cells of 10 different dogs and 10 different cats were used. Under standard conditions, cells were bred each with or without the influence of the Enerchip for 48 hours and the cell count, and thus the growth, was compared with one another. The increase in the stem cell proliferation due to the influence of the Enerchip was extremely clear and very pronounced. For the dog stem cells, an average growth of 26.2 percent was found in the group, in which the Enerchip Vitacell Dog was used. For the cats, an average growth increase of 35.92 percent was observed under the influence of the Enerchip Vitacell Cat.

In vivo, an increased proliferation of the stem cells would be advantageous for diseases with severe loss of tissue, for example heavy wear and tear of cartilage in the case of articulation problems, large cell death in the case of viral infections in the gastrointestinal tract or on respiratory mucous membranes for the cat cold complex. It is unclear whether the increased proliferation in vitro could be compared with a longer life in vivo.

With the second test arrangement, the vitality of stem cells in the culture was examined. In this process, the enzyme adenylate kinase was measured using bioluminescence. Adenylate kinase occurs in the interior of the cell and escapes in the case of cell damage or death of cells into the nutrient medium. High values of adenylate kinase therefore correspond to high damage values.

Without the influence of the Enerchip Vitacell Dog, an increase of the cell damage by 38.89% was measured for the dog stem cells. Without the influence of the Enerchip Vitacell Cat on the cat stem cells, an increase of the cell damage by 17.72 % was measured.

These values show the protection mechanism of the Enerchip on the viability and survivability of the stem cell.

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