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(54) **ENTRAPPED STEM CELLS AND USES THEREOF**

EINGEFANGENE STAMMZELLEN UND VERWENDUNGEN DAVON

CELLULES SOUCHES PIEGEES ET LEURS UTILISATIONS

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• **DANG ET AL.:** 'Controlled, scalable embryonic stem cell differentiation culture.' **STEM CELLS** vol. 22, 2004, pages 275 - 282, XP002362423
• **WEBER ET AL.:** 'Formation of cartilage matrix proteins by BMP-transfected murine mesenchymal stem cells encapsulated in a novel class of alginates.' **BIOMATERIALS** vol. 23, 2002, pages 2003 - 2013, XP004345246

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Description**FIELD OF THE INVENTION**

[0001] This invention relates to an in vitro method for inhibiting proliferation of at least a portion of a non-entrapped cell population, wherein said non-entrapped cell population was not derived using human embryos. The entrapped cells, when cultured in the entrapment material, produce a product which, when it is in contact with other non-entrapped, freely growing cells in vitro or in vivo, inhibits their proliferation. Further, the entrapment of the stem cells acts to inhibit the proliferation of at least some of the entrapped stem cells, and may inhibit the differentiation of at least a portion of the entrapped stem cells.

[0002] The invention further relates to:

the use of a medium for the manufacture of a medicament for treating cell proliferative disorders,
an in vitro method for inhibiting proliferation of a non-entrapped population of non-human embryonic stem cells, and
an in vitro method for inhibiting differentiation of at least a portion of a population of non-human embryonic stem cells.

BACKGROUND AND PRIOR ART

[0003] Entrapment of biological materials, such as cells, is a technique that has been used for various ends. Exemplary of the patent literature in this area are U.S. Patent Nos. 6,303,151 (Asina, et al.); 6,224,912 (Asina, et al.); 5,888,497 (Jain, et al.); 5,643,569 (Jain, et al.), and RE38,027 (Jain, et al.).

[0004] This family of related patents shows that cancer cells and islets can be entrapped in a biocompatible matrix, such as agarose, agarose/collagen mixtures, and agarose/gelatin mixtures, and then be coated with agarose. The resulting, entrapped cells produce materials which, inter alia, diffuse out of the permeable biocompatible matrices in which they are retained, and have useful biological properties. In the case of islets, insulin is produced. In the case of cancer cells, material diffuses from the matrix, and this material has an effect on the growth and proliferation of cancer cells. As review of the '912 and '151 patents, cited supra, will show, this effect crosses species, i.e., entrapped or encapsulated cancer cells from a given species produce material that inhibits the growth and/or proliferation of cancer cells from other species, as well as the species from which the cancer cells originated.

[0005] Additional examples of entrapment techniques include, e.g., U.S. Patent Nos. 5,227,298 (Weber, et al.); 5,053,332 (Cook, et al.); 4,997,443 (Walthall, et al.); 4,971,833 (Larsson, et al.); 4,902,295 (Walthall, et al.); 4,798,786 (Tice, et al.); 4,673,566 (Goosen, et al.); 4,647,536 (Mosbach, et al.); 4,409,331 (Lim); 4,392,909 (Lim); 4,352,883 (Lim); and 4,663,286 (Tsang, et al.).

[0006] Entrapment does not always result in a positive impact on the entrapped cells. For example, see Lloyd-George, et al., *Biomat. Art. Cells & Immob. Biotech.*, 21(3):323-333 (1993); Schinstine, et al., *Cell Transplant*, 41(1):93-102 (1995); Chi-cheportiche, et al., *Diabetologica*, 31:54-57 (1988); Jaeger, et al., *Progress In Braid Research*, 82:41-46 (1990); Zekorn, et al., *Diabetologica*, 29:99-106 (1992); Zhou, et al., *Am. J Physiol.*, 274:C13561362 (1998); Darquy, et al., *Diabetologica*, 28:776-780 (1985); Tse, et al., *Biotech. & Bioeng.*, 51:271-280 (1996); Jaeger, et al., *J Neurol.*, 21-469-480 (1992); Hortelano, et al., *Blood*, 87(12):5095-5103 (1996); Gardiner, et al., *Transp. Proc.*, 29:2019-2020 (1997).

[0007] None of the references discussed supra deals with the class of cells known as stem cells, including embryonic stem cells. US 2003/0119107 teaches the encapsulation of embryonic stem cells to form spheroids. Weber M et al., *Biomaterials*, vol. 23, p 2003-2013, 2002 describes encapsulation of cells from a mouse mesenchymal stem cell line in purified alginate. 1ml of 0.5 percent alginate is sufficient for a production of about 15,000 beads each containing approximately 50 cells. Further, Citrone P et al., *Human Gene Therapy*, vol. 13, p 1157 - 1166, 2002 discloses the use of microcapsules containing cells of a mouse myoblast cell line genetically modified to secrete interleukin 2 linked to a region of a humanized antibody with affinity to a tumor surface antigen. The encapsulated cells are used for the inhibition of tumor progression.

[0008] One definition of stem cells, advanced by Reya, et al., *Nature*, 414:105-111 (2001), refers to stems cells as cells which have the ability to perpetuate themselves through self renewal and to generate mature cells of particular tissues via differentiation. One can obtain different types of stem cells, including neural, hematolymphoid, myeloid, and other types of stem cells from various organs. These all have potential to develop into specific organs or tissues. Certain stem cells, such as embryonic stem cells, are pluripotent, in that their differentiation path has not been determined at all, and they can develop into various organs and tissues.

[0009] The discussions of the various therapeutic uses to which stem cells may be put are well known, and need not be discussed here. It is worth mentioning, as it bears on the invention described herein, that stem cells are very uncommon, their purification and separation from other cell types is laborious and difficult, and stem cells will differentiate into mature cell unless treated in some way to prevent this.

[0010] It has now been found that entrapment procedures, in line with those disclosed by Jain et al. and Iwata et al., Journ. Biomedical Material and Res., 26:967 (1992) affect stem cells in a very desirable way. To elaborate, entrapped stem cells produce materials which inhibit proliferation of various cell types, including stem cells and cancer cells. The effect of this material crosses species lines. Further, it has been found that stem cells, when entrapped as is described

herein, retain their differentiating abilities, including their pluripotentiality, for an indefinite period of time.

[0011] These features, as well as others, will be seen in the disclosure which now follows.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0012] According to the present invention, it is provided:

An in vitro method for inhibiting proliferation of at least a portion of a non-entrapped cell population according to claim 1,
A composition of matter of claim 1 for use in a method according to claim 8,

An in vitro method for inhibiting proliferation of a non-entrapped cell population according to claim 15,

Use of a medium for the manufacture of a medicament for treating cell proliferative disorders according to claim 20,
An in vitro method for inhibiting proliferation of a non-entrapped population of non-human embryonic stem cells according to claim 22,

An in vitro method for inhibiting proliferation of non-entrapped non-human embryonic stem cells according to claim 25, and

An in vitro method for inhibiting differentiation of at least a portion of a population of non-human embryonic stem cells according to claim 28.

EXAMPLE 1

[0013] Two different murine embryonic stem (ES) cell lines (i.e., ES-D3 and SCCPSA1, which are both publicly available) were obtained from the American Type Culture Collection ("ATCC").

[0014] Both lines were grown under standard culture conditions, which included growth as a monolayer, atop "STO" embryonic fibroblast feeder cells. These were also obtained from the ATCC. The stem cells were cultured in DMEM medium that had been supplemented with 100% ES-Qualified fetal bovine serum, leukemia inhibitory factor (LIF), and p-mercaptoethanol (collectively, "Medium A"). The cells, which were cryopreserved when received, were thawed, and established as cultures after at least 3 passages before being cultured as described, supra.

[0015] After three days, the ES cells were 70-80% confluent, and were trypsinized and then entrapped in agarose beads, coated with agarose, in accordance with US Patent Nos. 6,303,151; 6,224,912; and, 5,888,497, all of which are incorporated by reference. In brief, however, Sigma XII agarose was used, at an initial concentration of about 1.0%. A 100 μ l aliquot of this agarose solution was added to 34 μ l of cell suspension. The resulting beads contained $2.0 \times 10^5 \pm 1.5 \times 10^4$ murine embryonic stem cells. The beads were given a second coat of agarose, at a concentration of about 5.0%. The beads were cultured in medium as described supra, except no LIF or viable STO feeder cells were present ("Medium B").

[0016] The viability of cells in the beads over time was assessed, via standard histochemical and microscopic examination, as well as standard MTT assays, using cells removed from beads or maintained in the beads, at various points in time.

[0017] It was observed that entrapped stem cells increase their metabolic activity when first coated. This is followed by a decrease in activity, as cells die via apoptosis, reaching their lowest point of metabolic activity around day 21. After this low point, however, surviving cells slowly proliferate, and total metabolic activity was seen to gradually increase up to day 35 post entrapment and beyond. This parallels observations on entrapped cancer cells.

[0018] Morphologically, there was a significant difference between the colonies formed within the inner layer of agarose of the bead by the cancer cells and those formed by the stem cells. Although both types of colonies are ovoid in shape, those formed by the cancer cells are characterized by an outer zone of viable cells (generally two to three cells in thickness) with a central zone of eosinophilic cellular debris. The colonies formed by the stem cells, on the other hand, are fully occupied by viable cells and there is no central zone of cellular debris.

EXAMPLE 2

[0019] In these experiments, the inhibitory effect of stem cells on the proliferation of other stem cells was tested.

[0020] Ten-week-old agarose/agarose beads containing stem cells (SCC-PSA1 cells) were tested for viability using the MTT assay, discussed supra, and were cultured in Medium B discussed in example 1, for 6 days. After 6 days, the medium had been conditioned by the entrapped stem cells. It is therefore called the Stem-cell Conditioned Medium (SCM).

[0021] After these 6 days, the SCM was transferred to 6 well plates that contained fresh SCC-PSA1 cells. These plates

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each contained 9×10^5 STO feeder cells, which were covered with 1.5×10^4 SCC-PSA1 cells. The STO cells had been treated with mitomycin C to prevent proliferation. There were three controls, i.e., wells which contained Medium B (an unconditioned medium), and three wells that contained the SCM.

[0022] After 3 days, the contents of all wells were trypsinized, and total cells were counted, using standard methods. The raw count was adjusted to account for the 9×10^5 feeder cells. The results follow:

Test Article	Average Total Cells/Well	Standard Deviation	Cells After subtracting STO	Percent Inhibition (of SCC cells)
Control Medium	1.43×10^6	$\pm 9.9 \times 10^4$	5.27×10^5	
SCM (w/SCC)	1.19×10^6	$\pm 3.6 \times 10^4$	2.90×10^5	44.9%

[0023] A similar experiment was carried out, with the following results:

Test Article	Average Total Cells/Well	Standard Deviation	Cells After subtracting STO	Percent Inhibition (of SCC cells)
Control Medium	3.09×10^6	$\pm 1.7 \times 10^5$	1.41×10^6	
SCM (w/ SCC)	2.36×10^6	$\pm 9.5 \times 10^4$	6.88×10^5	51.4%

[0024] Further, the effect was not cell-line specific, as is demonstrated by the following results, where ES-D3 cells were added to the medium:

Test Article	Average Total Cells/Well	Standard Deviation	Cells After subtracting STO	Percent Inhibition (of ES-D3 cells)
Control Medium	1.27×10^6	$\pm 1.1 \times 10^5$	3.67×10^5	
SCM (w/SCC)	1.14×10^6	$\pm 7.6 \times 10^4$	2.37×10^5	35.5%

EXAMPLE 3

[0025] Example 2 showed that the proliferation inhibitory effect of the stem cells was not cell line specific. In the experiments described herein, the entrapped stem cells were tested for their ability to inhibit the proliferation of cancer cells.

[0026] In these experiments, RENCA tumor cells were used. A total of 15,000 tumor cells were seeded per well. SCM (conditioned either with SCC-PSAI or ES-D3), as described supra, was used, as was the control medium (Medium B), also as described.

[0027] With respect to the SCM, the conditioning took place over 5 days. The assay was run over a period of 32 weeks. The inhibition of the RENCA cells was determined by fixing the cells with 100% methanol, followed by staining with neutral red, lysis with SDS, and scanning with a spectrophotometer to measure the amount of neutral red in the cell lysate, which is proportional to the number of cells per well.

[0028] The results are summarized in the following two tables, which represent work with ES-D3, and SCC-PSA1 stem cells, respectively. The results for weeks 1-3 correlate with the results discussed in example 1, i.e., death of the entrapped stem cells, reaching a low point on day 21, followed by regeneration.

Week	1	3	12	16	20	24	28	32
% Inhibition of RENCA Cells by SCM (w/ES-D3)	-2.1%	-8.8%	39.0%	24.4%	25.0%	20.9%	34.9%	31.5%

Week	1	3	9	12	16	20	24	28	32
% Inhibition of RENCA Cells by SCM (w/SCC-PSA1)	-10.0%	8.9%	21.0%	40.4%	32.8%	22.5%	36.6%	38.0%	35.1%

EXAMPLE 4

[0029] In the preceding experiments, the ability of entrapped stem cells to inhibit proliferation of stem cells and cancer cells was tested, and proven. These next experiments were designed to determine if entrapped cancer cells could inhibit the proliferation of stem cells.

[0030] Stem cells were plated and cultured in the same way as was described, *supra*. RENCA cell containing beads, prepared as described in U.S. Patent Nos. 6,303,151; 6,224,912; and, 5,888,497 were cultured in Medium B to condition it, for 5 days. This RENCA Conditioned Medium (RCM) was then added to plated stem cells, and the stem cells were counted after 3 days. The results, which follow, present data for ES-D3 cells first, and then SCC-PSA1 cells:

Test Article	Average Total Cells/Well	Standard Deviation	Cells After subtracting STO	Percent Inhibition (of ES-D3)
Control Medium	1.69x10 ⁶	± 1.15x10 ⁴	7.93x10 ⁵	
RCM	1.42x10 ⁶	± 8.74x10 ⁴	5.23x10 ⁵	34.0%
Test Article	Average Total Cells/Well	Standard Deviation	Cells After subtracting STO	Percent Inhibition (of SCC-PSA1)
Control Medium	1.25x10 ⁶	± 8.08x10 ⁴	3.47x10 ⁵	
RCM	1.05x10 ⁶	± 4.04x10 ⁴	1.47x10 ⁵	57.7%

[0031] These results indicate that the entrapped cancer cells did inhibit the proliferation of stem cells.

EXAMPLE 5

[0032] One issue with stem cell research is the fact that, by their nature, stem cells differentiate. As it is difficult to secure stem cells and keep them from differentiating in the first place, it would be desirable to have a methodology available by which stem cells could be kept in their undifferentiated state, for as long a period as possible.

[0033] To this end, stem cells were entrapped as described in example 1, *supra*. The resulting structures were stored in Medium B described *supra*, and were tested over a period of more than two years.

[0034] Over this two-year period, stem cells were released from the entrapment structures and cultured under standard conditions (including STO co-cultures and LIP media additive). In all cases, the released cells established a traditional stem cell monolayer that proliferated in a non-differentiated manner, but maintained the capability to spontaneously differentiate. This demonstrates that the entrapment of stem cells can maintain their nondifferentiated phenotypes for greater than two years in the absence of the traditionally required inhibitors of differentiation (e.g., STO and LIF).

[0035] Notwithstanding this fact, if the cells do not receive the required materials after a short period of time, they do begin differentiation.

[0036] The foregoing examples describe the invention, which includes, inter alia, compositions of matter which can be used to produce material which suppresses proliferation of cells, such as, but not being limited to, cancer cells and stem cells, with the proviso that the stem cells are not human embryonic stem cells. These compositions comprise stem cells, such as non-human embryonic stem cells, entrapped in a selectively permeable material to form a structure which restricts the proliferation of the entrapped cells. As a result of their being restricted, the cells produce unexpectedly high amounts of material which suppresses proliferation of other cells. The restricted cells produce more of the material than comparable, non-restricted cells.

[0037] The material used to make the structures of the invention may include any biocompatible matter which restricts the growth of stem cells, thereby inducing them to produce greater amounts of cell proliferation growth-suppressing material. The structure has a suitable pore size such that the above material can diffuse to the external environment,

and such that it can prevent products or cells from the immune system of the host from entering the structure and causing the rejection of the cells or otherwise impair their ability to survive and continue to produce the desired material. The materials used to form the structure will also be capable of maintaining viable (proliferation-restricted, but surviving) cells both *in vitro* and *in vivo*, preferably for periods of up to several years, by providing for the entrance of proper nutrients, and elimination of cellular waste products, and a compatible physicochemical intrastructural environment. The resulting structures provide an environment suitable for the extended study of stem cells and their various differentiation, transcription and nuclear factors. Results therefrom can be used to direct the desired differentiation of other stem cells. The materials used to prepare the structure is preferably well tolerated when implanted *in vivo*, most preferably for the entire duration of implantation in the host.

[0038] A non-limiting list of materials and combinations of materials that might be utilized includes alginate-poly-(L-lysine); alginate-poly-(L-lysine)-alginate; alginate-poly-(L-lysine)-polyethyleneimine; chitosan-alginate; polyhydroxylethyl-methacrylate-methyl methacrylate; carbonylmethylcellulose; K-carragenan; chitosan; agarose-polyethersulphone-hexadi-methirine-bromide (Polybrene); ethyl-cellulose, silica gels; and combinations thereof.

[0039] The structures which comprise the compositions of matter may take many shapes, such as a bead, a sphere, a cylinder, a capsule, a sheet or any other shape which is suitable for implantation in a subject, and/or culture in an *in vitro* milieu. The size of the structure can vary, depending upon its eventual use, as will be clear to the skilled artisan.

[0040] The structures of the invention are selectively permeable, such that nutrients may enter the structure, and so that the proliferation-inhibiting material as well as cellular waste may leave the structure. For *in vivo* use, it is preferred that the structures prevent the entry of products or cells of the immune system of a host which would cause the rejection of the cells, or otherwise impair the ability of the cells to produce the proliferation-suppressive material.

[0041] "Entrapped" as used herein means that the cells are contained within a structure which prevents their escape to the environment surrounding the structure, be that an *in vitro* or *in vivo* environment. Notwithstanding the inability to escape therefrom, the cells are within a structure which both permits entry of molecules such as water, nutrients, and so forth, and permits the passage from the structure of waste materials and molecular products produced by the cells. The structure in which the cells are contained thus supports the continued viability/survival of the cells for long periods of time. It may also, depending on the nature of the structure/material, cause the cells contained within it to alter their behavior, including, but not limited to, such behavior as proliferation, state of differentiation and/or phenotypic expression. By inhibiting differentiation, one *de facto* has a storage device useful for maintaining stem cells as stem cells. Exemplary, but non-exclusive, means of entrapping the cells include encapsulating them, encasing them, enclosing them, or otherwise surrounding them on all sides with some permeable material. Via the entrapment, the proliferation of the entrapped stem cells is inhibited. Further, there are situations where at least a portion of the population that is entrapped does not undergo any differentiation as well.

[0042] Another aspect of the invention includes compositions which are useful in suppressing cell proliferation. The compositions are prepared by culturing restricted cells as described supra in an appropriate culture medium, followed by recovery of the resultant conditioned medium. Concentrates can then be formed from the conditioned medium.

[0043] The invention is not limited to any particular type of stem cell species, with the proviso that human embryonic stem cells are excluded; any stem cell type except human embryonic stem cells may be used in accordance with the invention. Exemplary types of cells which can be used are murine stem cells, as well as stem cells from other species, especially mammalian species. Non-human embryonic stem cells are especially preferred, but stem cells obtained from various organs and/or organ systems may be used as well.

[0044] As will be clear from this disclosure, a further aspect of the invention is therapeutic methods for treating individuals suffering from cell proliferation disorders such as polycystic kidney disease, hypertrophic tissue reaction (including scar formation), autoimmune disease, lympho-proliferative disorders, polycythemia vera, as well as both benign and malignant cell neoplasia. When used in a therapeutic context, as will be elaborated upon *infra*, the type of cell restricted in the structure need not be the same type of cell that is causing the disorder from which the individual is suffering, although it can be. One such method involves inserting at least one of the structures of the invention into the subject, in an amount sufficient to cause suppression of cell proliferation in the subject. Preferably, the subject is a human being, although it is applicable to other animals, such as domestic animals, farm animals, or any type of animal.

[0045] The composition of the present invention can be used as primary therapy in the treatment of various cell proliferative disorders, and as an adjunct treatment in combination with other therapies. For example, in neoplastic disorders, such as cancer, patients may be treated with compositions and methods described herein, in conjunction with radiation therapy, chemotherapy, or treatment with other biologically active materials such as cytokines, anti-sense molecules, steroid hormones, gene therapy, and the like. Additionally, the compositions and methods of the invention can be used in conjunction with surgical procedures to treat disorders such as cancer, e.g., by implanting the structures after resection of a tumor to prevent regrowth and metastases. Cancers which are present in an inoperable state may be rendered operable by treatment with the anti-proliferative compositions of the invention. The excess proliferation of cells that are not needed or desirable for proper organ system function, but are not neoplastic, such as that of polycythemia vera or polycystic kidney disease, may also be treated by this means. Hyperproliferative disorders, such as polycythemia

vera and polycystic kidney disease, involve cells that exhibit excess proliferation but generate otherwise normal (i.e., non-neoplastic or transformed) cells. Such disorders, resulting in numerous cells that are not needed or desirable for proper organ function, may also be treated by these means. Additionally, conditions which are characterized by hyperproliferative, normal cells, such as hypertrophic scars, can also be treated in this way. In conditions such as this one, normal cells, i.e., fibroblasts have proliferated beyond what is necessary for healing, but unlike neoplasias, they are not characterized by further, ongoing, unregulated proliferation. Other conditions characterized by this phenomenon well known to the skilled artisan, and need not be set forth here.

[0046] The compositions of the invention can also be used prophylactically in individuals at risk for developing cell proliferation disorders, subjects who show the presence of individual risk factors, a family history of the disorder generally, family history of a specific type (e.g., breast cancer), and exposure to occupational or other problematic materials. For prophylaxis against cancer, e.g., a prophylactically effective amount of the structures of the invention are administered to the individual upon identification of one or more risk factors.

[0047] As indicated by the examples, supra, the antiproliferative effect is not limited by the type of cell used, nor by the species from which the stem cell originated. Hence, one can administer structures which contain stem cells of a first type to a subject of a different species. For example, murine stem cells may be restricted in the structure of the invention, and then be administered to a human. Of course, the structures may contain stem cells from the same species as is being treated. Still further, the stem cell may be taken from the individual to be treated, entrapped and restricted, and then administered to the same individual.

[0048] Processes for making the structures of the invention are also a part of the invention.

Claims

1. An in vitro method for inhibiting proliferation of at least a portion of a non-entrapped cell population, comprising culturing said cell population in the presence of a composition of matter comprising a sample of non-human embryonic stem cells entrapped in a biocompatible, selectively permeable structure, wherein entrapment of said sample of non-human embryonic stem cells inhibits proliferation of at least a portion of the entrapped non-human embryonic stem cells wherein said non-entrapped cell population was not derived using human embryos.
2. The in vitro method of claim 1, wherein said cell population is a stem cell population with the proviso that it is not a human embryonic stem cell population.
3. The in vitro method of claim 1, wherein said cell population is from a species different from the species of origin of the cells entrapped in said composition of matter, or wherein said cell population is from the same species as is the species of origin of the cells entrapped in said composition of matter.
4. The in vitro method of claim 1, wherein said cell population is a cancer cell population.
5. The in vitro method of claim 1, wherein said cell population is a mammalian cell population.
6. The in vitro method of claim 1, wherein said cell population is a hyperproliferative cell population.
7. The in vitro method of claim 5, wherein said mammalian cell population is a human cell population, or a murine cell population.
8. A composition of matter comprising a sample of non-human embryonic stem cells entrapped in a biocompatible, selectively permeable structure, wherein entrapment of said sample of non-human embryonic stem cells inhibits proliferation of at least a portion of the entrapped non-human embryonic stem cells, for use in a method of treatment of cell proliferative disorders.
9. The use of claim 8, wherein said cell population is a stem cell population, or wherein said cell population comprises neoplastic cells, or wherein said cell population is a hyperproliferative cell population.
10. the use of claim 8, wherein cells entrapped in said composition of matter are from a species different from said subject.
11. The use of claim 8, wherein cells entrapped in said composition of matter are from the same species as said subject.
12. The use of claim 11, wherein said cells are autologous to said subject.

13. The use of claim 8, wherein said subject is a human.

14. The use of claim 11, wherein said subject is a human.

15. An in vitro method for inhibiting proliferation of a non-entrapped cell population, comprising culturing a composition of matter comprising a sample of non-human embryonic stem cells entrapped in a biocompatible, selectively permeable structure, wherein entrapment of said sample of non-human embryonic stem cells inhibits proliferation of at least a portion of the entrapped non-human embryonic stem cells, in a medium for a time sufficient to permit diffusion of a cell proliferation inhibiting material into said medium, and contacting said medium to said non-entrapped cell population for a time sufficient to inhibit proliferation of said non-entrapped cell population, wherein said non-entrapped cell population was not derived using human embryos.

16. The in vitro method of claim 15, wherein said non-entrapped cell population is a stem cell population with the proviso that it is not a human embryonic stem cell population.

17. The in vitro method of claim 15, wherein said non-entrapped cell population and the cells entrapped in said composition of matter are from different species.

18. The in vitro method of claim 15, wherein said non-entrapped cell population and the cells entrapped in said composition of matter are from the same species.

19. The in vitro method of claim 16, wherein said stem cell population is a mammalian cell population, preferably a human cell population, or a murine cell population.

20. Use of a medium for the manufacture of a medicament for treating cell proliferative disorders wherein the medium is obtainable by culturing the composition of matter comprising a sample of non-human embryonic stem cells entrapped in a biocompatible, selectively permeable structure, wherein entrapment of said sample of non-human embryonic stem cells inhibits proliferation of at least a portion of the entrapped non-human embryonic stem cells in a medium for a time sufficient to permit diffusion of a cell proliferation inhibiting material into said medium.

21. Use of claim 20, wherein said cell population is a stem cell population, or wherein said cell population comprises neoplastic cells.

22. An in vitro method for inhibiting proliferation of a non-entrapped population of non-human embryonic stem cells comprising culturing said stem cells in the presence of a composition of matter which comprises a sample of cancer cells entrapped in a biocompatible, selectively permeable structure, wherein said sample of cancer cells produces material which inhibits proliferation of said stem cells.

23. The method of claim 22, wherein said stem cells are mammalian stem cells, preferably murine stem cells.

24. The method of claim 22, wherein said stem cells and cancer cells originate from the same species, or wherein said stem cells and cancer cells originate from different species.

25. An in vitro method for inhibiting proliferation of non-entrapped non-human embryonic stem cells, comprising culturing a composition of matter which comprises a sample of cancer cells entrapped in a biocompatible, selectively permeable structure, wherein said sample of cancer cells produces material which inhibits proliferation of stem cells and which diffuses into a culture medium, and contacting said culture medium to said stem cells.

26. The in vitro method of claim 25, wherein said stem cells are mammalian stem cells, preferably murine stem cells.

27. The in vitro method of claim 25, wherein said stem cells and cancer cells originate from the same species, or wherein said stem cells and cancer cells originate from different species.

28. An in vitro method for inhibiting differentiation of at least a portion of a population of non-human embryonic stem cells, comprising entrapping said non-human embryonic stem cells in a biocompatible, selectively permeable structure, wherein such entrapment causes at least a portion of said embryonic stem cells to remain in an undifferentiated state.

Patentansprüche

- 5 1. In vitro-Verfahren zur Inhibierung der Proliferation von wenigstens einem Teil einer nicht eingeschlossenen Zellpopulation, umfassend die Kultivierung der Zellpopulation in der Gegenwart einer Stoffzusammensetzung, welche eine in einer biokompatiblen, selektiv permeablen Struktur eingeschlossen Probe von nicht-menschlichen embryonalen Stammzellen umfasst, wobei der Einschluss der Probe von nicht-menschlichen embryonalen Stammzellen die Proliferation von wenigstens einem Teil der eingeschlossenen nicht-menschlichen embryonalen Stammzellen verhindert, wobei die nicht eingeschlossene Zellpopulation nicht unter Verwendung von menschlichen Embryonen abgeleitet wurde.
- 10 2. In vitro Verfahren nach Anspruch 1, wobei die Zellpopulation eine Stammzellpopulation ist, mit der Maßgabe, dass es keine menschliche embryonale Stammzellpopulation ist.
- 15 3. In vitro Verfahren nach Anspruch 1, wobei die Zellpopulation von einer anderen Spezies stammt als die Ursprungsspezies in der der Stoffzusammensetzung eingeschlossenen Zellen, oder, wobei die Zellpopulation von der gleichen Spezies ist wie die Ursprungsspezies der Zellen, die in der Stoffzusammensetzung eingeschlossen sind.
- 20 4. In vitro Verfahren nach Anspruch 1, wobei die Zellpopulation eine Krebszellpopulation ist.
- 25 5. In vitro Verfahren nach Anspruch 1, wobei die Zellpopulation eine Säugetierzellpopulation ist.
6. In vitro Verfahren nach Anspruch 1, wobei die Zellpopulation eine hyperproliferative Zellpopulation ist.
7. In vitro Verfahren nach Anspruch 5, wobei die Säugetierzellpopulation eine menschliche Zellpopulation oder eine Mauszellpopulation ist.
- 30 8. Stoffzusammensetzung, umfassend eine Probe von nicht-menschlichen embryonalen Stammzellen, welche in einer biokompatiblen, selektiv permeablen Struktur eingeschlossen sind, wobei der Einschluss der Probe von nicht-menschlichen embryonalen Stammzellen die Proliferation von wenigstens einem Teil der eingeschlossenen nicht-menschlichen embryonalen Stammzellen inhibiert, zur Verwendung in einem Verfahren zur Behandlung von Zellwachstumsstörungen.
- 35 9. Verwendung nach Anspruch 8, wobei die Zellpopulation eine Stammzellpopulation ist, oder, wobei die Zellpopulation neoplastische Zellen umfasst, oder, wobei die Zellpopulation eine hyperproliferative Zellpopulation ist.
- 40 10. Verwendung nach Anspruch 8, wobei die in der Stoffzusammensetzung eingeschlossenen Zellen von einer anderen Spezies als das Subjekt sind.
11. Verwendung nach Anspruch 8, wobei die in der Stoffzusammensetzung eingeschlossenen Zellen von der gleichen Spezies wie das Subjekt sind.
- 45 12. Verwendung nach Anspruch 11, wobei die Zellen von dem Subjekt selbst stammen.
13. Verwendung nach Anspruch 8, wobei das Subjekt ein Mensch ist.
14. Verwendung nach Anspruch 11, wobei das Subjekt ein Mensch ist.
- 50 15. In vitro-Verfahren zur Inhibierung der Proliferation einer nicht eingeschlossenen Zellpopulation umfassend das Kultivieren einer Stoffzusammensetzung, welche eine Probe von nicht-menschlichen embryonalen Stammzellen umfasst, die in einer biokompatiblen, selektiv permeablen Struktur eingeschlossen sind, wobei der Einschluss der Probe von nicht-menschlichen embryonalen Stammzellen das Wachstum von wenigstens einem Teil der eingeschlossenen nicht-menschlichen embryonalen Stammzellen inhibiert, in einem Medium für eine ausreichende Zeit, um die Diffusion von zellwachstumsinhibierendem Material in das Medium zuzulassen, und das Kontaktieren des Mediums mit der nicht eingeschlossenen Zellpopulation für eine ausreichende Zeit, um das Wachstum von der nicht eingeschlossenen Zellpopulation zu inhibieren, wobei die nicht eingeschlossene Zellpopulation nicht unter Verwendung von menschlichen Embryonen abgeleitet wurde.
- 55 16. In vitro Verfahren nach Anspruch 15, wobei die nicht eingeschlossene Zellpopulation eine Stammzellpopulation ist,

mit der Maßgabe, dass es sich nicht um eine menschliche Stammzellpopulation handelt.

17. In vitro Verfahren nach Anspruch 15, wobei die nicht eingeschlossene Zellpopulation und die in der Stoffzusammensetzung eingeschlossenen Zellen von unterschiedlichen Spezies sind.

18. In vitro Verfahren nach Anspruch 15, wobei die nicht eingeschlossene Zellpopulation und die in der Stoffzusammensetzung eingeschlossenen Zellen von der gleichen Spezies sind.

19. In vitro Verfahren nach Anspruch 16, wobei die Stammzellpopulation eine Säugetierzellpopulation, bevorzugt eine menschliche Zellpopulation oder eine Mauszellpopulation, ist.

20. Verwendung eines Mediums zur Herstellung eines Medikaments zur Behandlung von Zellproliferationsstörungen, wobei das Medium durch die Kultivierung einer Stoffzusammensetzung, welche eine Probe von nicht menschlichen embryonalen Stammzellen umfasst, die in einer biokompatiblen selektiv permeablen Struktur eingeschlossen sind, wobei der Einschluss der Probe von nicht-menschlichen embryonalen Stammzellen die Proliferation von wenigstens einem Teil der eingeschlossenen nicht-menschlichen embryonalen Stammzellen inhibiert, in einem Medium für eine ausreichende Zeit, um die Diffusion von einem Zellwachstumsinhibierenden Material in das Medium zu ermöglichen, erhältlich ist.

21. Verwendung nach Anspruch 20, wobei die Zellpopulation eine Stammzellpopulation ist, oder, wobei die Zellpopulation neoplastische Zellen umfasst.

22. In vitro-Verfahren zur Inhibierung der Proliferation einer nicht eingeschlossenen Population von nicht menschlichen embryonalen Stammzellen, welches die Kultivierung der Stammzellen in der Gegenwart von einer Stoffzusammensetzung umfasst, welche eine Probe von Krebszellen umfasst, welche in einer biokompatiblen, selektiv permeablen Struktur eingeschlossen sind, wobei die Probe von Krebszellen Material erzeugt, welches die Proliferation von Stammzellen inhibiert.

23. Verfahren nach Anspruch 22, wobei die Stammzellen Säugetier-Stammzellen und bevorzugt Mausstammzellen sind.

24. Verfahren nach Anspruch 22, wobei die Stammzellen und Krebszellen von der gleichen Spezies stammen, oder, wobei die Stammzellen und Krebszellen von unterschiedlichen Spezies stammen.

25. In vitro-Verfahren zur Inhibierung der Zellproliferation von nicht eingeschlossenen nicht-menschlichen embryonalen Stammzellen umfassend die Kultivierung einer Stoffzusammensetzung, welche eine in einer biokompatiblen, selektiv permeablen Struktur eingeschlossene Probe von Krebszellen umfasst, wobei die Probe von Krebszellen Material erzeugt, welches die Zellproliferation von Stammzellen inhibiert, und, welches in ein Wachstumsmedium diffundiert, sowie das Kontaktieren des Wachstumsmediums mit den Stammzellen.

26. In vitro-Verfahren nach Anspruch 25, wobei die Stammzellen Säugetier-Stammzellen, bevorzugt Mausstammzellen, sind.

27. In vitro-Verfahren nach Anspruch 25, wobei die Stammzellen und die Krebszellen von der gleichen Spezies stammen, oder, wobei die Stammzellen und die Krebszellen von unterschiedlichen Spezies stammen.

28. In vitro-Verfahren zur Inhibierung der Differenzierung von wenigstens einem Teil einer Population von nicht-menschlichen embryonalen Stammzellen umfassend das Einschließen nicht-menschlicher embryonaler Stammzellen in einer biokompatiblen, selektiv permeablen Struktur, wobei ein solcher Einschluss dazu führt, dass wenigstens eine Teil der embryonalen Stammzellen in einem undifferenzierten Zustand verbleibt.

Revendications

1. Procédé in vitro d'inhibition de la prolifération d'au moins une partie d'une population de cellules non piégées, comprenant la culture de ladite population de cellules en présence d'une composition de matière comprenant un échantillon de cellules souches embryonnaires non humaines piégées dans une structure biocompatible sélectivement perméable, dans lequel le piégeage dudit échantillon de cellules souches embryonnaires non humaines inhibe la prolifération d'au moins une partie des cellules souches embryonnaires non humaines piégées, dans lequel ladite

population de cellules non piégées n'a pas été dérivée en utilisant des embryons humains.

2. Procédé in vitro selon la revendication 1, dans lequel ladite population de cellules est une population de cellules souches à condition qu'il ne s'agisse pas d'une population de cellules souches embryonnaires humaines.
3. Procédé in vitro selon la revendication 1, dans lequel ladite population de cellules est d'une espèce différente de l'espèce d'origine des cellules piégées dans ladite composition de matière, ou dans lequel ladite population de cellules est de la même espèce que l'espèce d'origine des cellules piégées dans ladite composition de matière.
4. Procédé in vitro selon la revendication 1, dans lequel ladite population de cellules est une population de cellules cancéreuses.
5. Procédé in vitro selon la revendication 1, dans lequel ladite population de cellules est une population de cellules mammaliennes.
6. Procédé in vitro selon la revendication 1, dans lequel ladite population de cellules est une population de cellules hyperprolifératives.
7. Procédé in vitro selon la revendication 5, dans lequel ladite population de cellules mammaliennes est une population de cellules humaines, ou une population de cellules murines.
8. Composition de matière comprenant un échantillon de cellules souches embryonnaires non humaines piégées dans une structure biocompatible sélectivement perméable, dans laquelle le piégeage dudit échantillon de cellules souches embryonnaires non humaines inhibe la prolifération d'au moins une partie des cellules souches embryonnaires non humaines piégées, pour son utilisation dans un procédé de traitement de troubles de la prolifération cellulaire.
9. Utilisation selon la revendication 8, dans laquelle ladite population de cellules est une population de cellules souches, ou dans laquelle ladite population de cellules comprend des cellules néoplasiques, ou dans laquelle ladite population de cellules est une population de cellules hyperprolifératives.
10. Utilisation selon la revendication 8, dans laquelle les cellules piégées dans ladite composition de matière sont d'une espèce différente de celle dudit sujet.
11. Utilisation selon la revendication 8, dans laquelle les cellules piégées dans ladite composition de matière sont de la même espèce que ledit sujet.
12. Utilisation selon la revendication 11, dans laquelle les cellules sont autologues au dit sujet.
13. Utilisation selon la revendication 8, dans laquelle ledit sujet est un être humain.
14. Utilisation selon la revendication 11, dans laquelle ledit sujet est un être humain.
15. Procédé in vitro d'inhibition de la prolifération d'une population de cellules non piégées, comprenant la culture d'une composition de matière comprenant un échantillon de cellules souches embryonnaires non humaines piégées dans une structure biocompatible sélectivement perméable, dans lequel le piégeage dudit échantillon de cellules souches embryonnaires non humaines inhibe la prolifération d'au moins une partie des cellules souches embryonnaires non humaines piégées, dans un milieu pendant une durée suffisante pour permettre la diffusion d'un matériau d'inhibition de la prolifération cellulaire dans ledit milieu, et la mise en contact dudit milieu avec ladite population de cellules non piégées pendant une durée suffisante pour inhiber la prolifération de ladite population de cellules non piégées, dans lequel ladite population de cellules non piégées n'a pas été dérivée en utilisant des embryons humains.
16. Procédé in vitro selon la revendication 15, dans lequel ladite population de cellules non piégées est une population de cellules souches à condition qu'il ne s'agisse pas d'une population de cellules souches embryonnaires humaines.
17. Procédé in vitro selon la revendication 15, dans lequel ladite population de cellules non piégées et les cellules piégées dans ladite composition de matière sont d'espèces différentes.
18. Procédé in vitro selon la revendication 15, dans lequel ladite population de cellules non piégées et les cellules

piégées dans ladite composition de matière sont de la même espèce.

19. Procédé in vitro selon la revendication 16, dans lequel ladite population de cellules souches est une population de cellules mammaliennes, de préférence une population de cellules humaines, ou une population de cellules murines.

20. Utilisation d'un milieu pour la fabrication d'un médicament pour le traitement des troubles de la prolifération cellulaire, dans laquelle le milieu peut être obtenu par culture de la composition de matière comprenant un échantillon de cellules souches embryonnaires non humaines piégées dans une structure biocompatible sélectivement perméable, dans laquelle le piégeage dudit échantillon de cellules souches embryonnaires non humaines inhibe la prolifération d'au moins une partie des cellules souches embryonnaires non humaines piégées dans un milieu pendant une durée suffisante pour permettre la diffusion d'un matériau d'inhibition de la prolifération cellulaire dans ledit milieu.

21. Utilisation selon la revendication 20, dans laquelle ladite population de cellules est une population de cellules souches, ou dans laquelle ladite population de cellules comprend des cellules néoplasiques.

22. Procédé in vitro d'inhibition de la prolifération d'une population de cellules souches embryonnaires non humaines non piégées comprenant la culture desdites cellules souches en présence d'une composition de matière qui comprend un échantillon de cellules cancéreuses piégées dans une structure biocompatible sélectivement perméable, dans lequel ledit échantillon de cellules cancéreuses produit un matériau qui inhibe la prolifération desdites cellules souches.

23. Procédé selon la revendication 22, dans lequel lesdites cellules souches sont des cellules souches mammaliennes, de préférence des cellules souches murines.

24. Procédé selon la revendication 22, dans lequel lesdites cellules souches et lesdites cellules cancéreuses proviennent de la même espèce, ou dans lequel lesdites cellules souches et lesdites cellules cancéreuses proviennent d'espèces différentes.

25. Procédé in vitro d'inhibition de la prolifération de cellules souches embryonnaires non humaines, non piégées, comprenant la culture d'une composition de matière qui comprend un échantillon de cellules cancéreuses piégées dans une structure biocompatible sélectivement perméable, dans lequel ledit échantillon de cellules cancéreuses produit un matériau qui inhibe la prolifération des cellules souches et qui se diffuse dans un milieu de culture, et la mise en contact dudit milieu de culture avec lesdites cellules souches.

26. Procédé in vitro selon la revendication 25, dans lequel lesdites cellules souches sont des cellules souches mammaliennes, de préférence des cellules souches murines.

27. Procédé in vitro selon la revendication 25, dans lequel lesdites cellules souches et lesdites cellules cancéreuses proviennent de la même espèce, ou dans lequel lesdites cellules souches et lesdites cellules cancéreuses proviennent d'espèces différentes.

28. Procédé in vitro d'inhibition de la différenciation d'au moins une partie d'une population de cellules souches embryonnaires non humaines, comprenant le piégeage desdites cellules souches embryonnaires non humaines dans une structure biocompatible sélectivement perméable, dans lequel ledit piégeage entraîne le fait qu'au moins une partie desdites cellules souches embryonnaires restent dans un état non différencié.

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