

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**UTILITY PATENT APPLICATION FOR:****THREE-DIMENSIONAL PHYSIOLOGICAL
MATRICES FOR ONCOLOGICAL TESTING,
AND METHODS FOR THEIR PRODUCTION AND USE**

Inventors: Penelope Catherine Costello
Warren Bruce McDonald

PRIORITY STATEMENT

[0001] Applicants hereby claim priority to U.S. Provisional Patent Application Serial No. 60/813,689, filed June 15, 2006, and to U.S. Provisional Patent Application Serial No. _____ (TBD), filed June 13, 2007, under Attorney Reference No. 85593-200971, and entitled Three-Dimensional Physiological Matrices, Methods, Apparatus And Kits For The Expedited Design, Evaluation, Data Organization And Reporting With Respect To Oncological Remedies And Therapies. All the materials and information provided in the above-identified applications are hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to matrices, methods, apparatus and kits for the expedited design and evaluation of oncological remedies. Key aspects of the inventions include matrices, and especially gel matrices, which are adapted and arranged to provide conditions which permit behaviors, such as the movement of cells from a sample of target tissue, to be evaluated in a manner that produces data useful for evaluating the oncological status of the cells. In a further key aspect, the invention permits the in vitro testing and analyses of one or more therapies, such as therapeutic pharmaceutical compounds, or radiological therapies, with respect to specific target tissues and cells removed from a patient.

BACKGROUND OF THE INVENTION

[0003] Cancer is the loss of control of one or more of the regulatory systems which regulate the growth of cells and tissues. An uncontrolled growth of a particular tissue or cell type is a specific type of cancer. There are many types of cancer. In the treatment of

cancer, one or more therapeutic remedies are typically used in attempts to cure the disease or ameliorate its effects. Many differences exist between classes of tumors and, indeed, individual tumors of the same type. There are many possible therapies for cancers in general. Nonetheless, most therapies do not work, or do not work to the extent necessary to provide the degree of cure, remediation or desired palliative effect for a specific cancer. Because of this, it is sometimes difficult, time-consuming and expensive to attempt to determine what therapeutic compounds may be effective to treat a particular tumor or cancer type. Oncologists and other physicians therefore often choose a therapy based on little or no pragmatic information regarding the specific tumor. In effect, their determinations of which therapy or therapies to employ are often guesses. It is therefore very important to be able to more effectively match a specific effective therapy to a specific tumor, and to be able to do so in a reasonable amount of time. As a consequence of these needs, many technologies have attempted to address these needs, but none have been acceptably successful.

[0004] Various experiments have been directed toward measuring the effects of certain therapies on malignant cells in vitro. One of these attempts is shown in "Effects of Radiation on a Three-Dimensional Model of Malignant Glioma Invasion" International Journal of Devl. Neuroscience, Vol. 17, issue 5-6, 643-651, August 1999, (Bauman *et al.*). The Bauman *et al.* researchers used suspensions of an established cell line, the C6 astrocytoma line, to show that the three-dimensional migration of known, cultured malignant cells into a collagen matrix could be observed. The malignant glioma cells thus cultured, disrupted and processed into cloned spheroids, which were then implanted into a gel matrix, and then subjected to one or more doses of fractionated radiation. Changes in the distance of invasion in response to single dose and fractionated radiation were measured over a period of 5 days.

[0005] Similar experiments were reported in "Effects Of Radiation On A Model Of Malignant Glioma Invasion", Journal of Neuro-Oncology 44: 223-231, 1999, (Baumann *et al.*). In this reference, the Baumann *et al.* researchers used the same C6 cell line and experimental protocols to test BCNU and dexamethasone, and to compare these results with those of radiation dosing on the transformed cells.

[0006] Significantly, in both series of experiments reported by Bauman *et al.*, the cloned C6 astrocytoma cells were subjected to disruption by trypsinization, and also subjected to centrifugal forces for 3-4 weeks in spinner flasks. Thus, the cells of Bauman *et al.* were already known to be transformed to a great extent, were generations removed from

the original tissue, required disruptive chemical processing, and were a subset of cloned malignant cells at the time they were subject to radiation doses. C6 astrocytoma cells were maintained in tissue culture as cloned representatives of malignant rat glioma cells. Indeed, the cells of the Bauman *et al.* experiments were removed and established as a cell line years before they were subjected to the experiments of Bauman *et al.*

[0007] In significant contrast, the present invention uses a sample quantity of fresh tissue taken directly from an animal, such as a human patient. Significantly, the samples used in the present invention are not disrupted by trypsin or other enzymes, but are mechanically divided into sample portions of appropriate sizes for testing. Thus, the present methods maintain the cell-to-cell contact of the sample tissue as if it were still in vivo. An additional difference pertains to the fact that the experiments of Bauman *et al.* used an established cell line, that is, cells that were already known to have been transformed long before, and in an unknown way, to an extent great enough that they could be used to establish a tissue culture cell line. One could not therefore expect the cells of Baumann *et al.* to behave in a manner reasonably replicative of fresh or in vivo cells.

[0008] Others have attempted to provide ways of evaluating the response of tumor cells to chemotherapeutics. In U.S. Patent No. 5,242,806 to Yen-Maguire *et al.*, entitled Method For Conducting The Cytotoxicity Assays On Tumor Cells, a "growth matrix" of bovine cornea endothelium cells is sometimes employed as a coating in the wells of multi-well plates in order to facilitate the attachment of cells to the plate surfaces. Essentially, Yen-McGuire discloses ways of culturing cellular suspensions which have been grown in two dimensions, and then assaying the responses of the processed cells to various cytotoxic or chemotherapeutic compounds.

[0009] The cellular suspensions of Yen McGuire are provided with a defined, selective growth medium which is designed and formulated to promote the growth of epithelial tumor cells while inhibiting the growth of normal cells. Yen-McGuire thus teaches the use of selective nutrition to skew cellular growth and behavior. Indeed, the relative amount of cellular growth is measured to provide information regarding the sensitivity of the highly processed tumor cells. Thus, Yen-McGuire does not provide any analysis with respect to an original tumor sample or fragment thereof, nor does it comprehend the advantages of an in vitro system which replicates significant aspects of the three-dimensional environment of tumor tissue in vivo.

[0010] These problems regarding the lack of analytic tools which are effectively usable to provide information specific to particular tumors, combined with the fact that cancer often progresses rapidly, have created a significant need for means and methods to quickly obtain information useful for testing and evaluating specific therapies, for example, therapeutic compounds, to determine their effectiveness with respect to particular tumors or cancer types. There is thus a significant need for such means and methods.

SUMMARY

[0011] Accordingly, it is an object of the present disclosure to remedy the above-mentioned drawbacks by providing three-dimensional matrices, such as gels, as well as methods and kits for evaluating and testing tissue and cells to determine if they are abnormal, pre-cancerous, or cancerous.

[0012] Another object of the present disclosure is to provide methods and kits for testing one or more therapeutic substances to determine their efficacy against a particular tumor or type of tumor.

[0013] In accordance with these and other objects, and in accordance with the context of the present disclosure with respect to providing novel and non-obvious improvements in all relevant fields, multiple means and methods of practicing the present disclosures are provided.

[0013a] According to a first aspect, there is provided a method for evaluating the efficacy of one or more therapeutic compounds with respect to at least one tissue from an animal, said tissue comprising one or more types of cells, and said tissue being suspected of being abnormal, cancerous or pre-cancerous, said method comprising the steps of A) implanting a sample quantity of said suspected tissue at least partially within a three-dimensional physiological matrix, wherein said tissue is essentially completely submerged within said matrix, said matrix being adapted and arranged for measuring one or more parameters of the behavior of said tissue and said cells, B) providing sufficient nutrition to said cells and said tissue so that said parameters can be measured, C) providing at least one of said one or more therapeutic compounds to said cells and said tissue, D) incubating or culturing said tissue and said cells with said one or more therapeutic compounds in an environment suitable for the growth of said cells and said tissue for a time sufficient to obtain measurements with respect to said one or more parameters, and E) measuring one or more of said parameters to obtain data regarding said behaviors with respect to said one or more therapeutic compounds.

[0013b] According to a second aspect, there is provided a method for evaluating the efficacy of one or more therapies with respect to at least one tissue from an animal, said tissue comprising one or more types of cells, and said tissue being suspected of being abnormal, pre-cancerous or cancerous, said method comprising the steps of A) implanting a sample of tissue from said animal at least partially within a three-dimensional physiological matrix, wherein said tissue is essentially completely submerged within said matrix, said matrix being adapted and arranged for measuring one or more parameters of the behavior of said cells of said tissue or cells, B) providing sufficient nutrition to said cells of said tissue of said portions so that said parameters can be measured, C) subjecting said cells and said tissue to at least one of said one or more therapies D) incubating or culturing said tissue and said cells of said portions in an environment suitable for the growth of said cells and said tissue for a time sufficient to obtain measurements with respect to said one or more parameters for each of said portions, and E) measuring, with respect to each of said portions, one or more of said parameters to obtain data regarding said behaviors with respect to said one or more therapies.

[0014] In accordance with these and other objects, means and methods for evaluating the oncological characteristics of at least one tissue or cells from an animal, such as a human being, which cells or tissue are suspected of being abnormal, cancerous or pre-cancerous, are provided. In one preferred embodiment, a method of the present disclosure comprises the steps of A) obtaining a sample quantity of the suspected tumor tissue or cells from the animal, wherein the tissue comprises one or more types of cells, B) implanting at least part of the sample of the suspected tumor tissue at least partially within a three-dimensional physiological matrix, the matrix being adapted and arranged for measuring one or more parameters of the behavior of the cells of the suspected tumor tissue, C) providing sufficient nutrition to the cells of the tissue or cells so that the parameters can be measured, D) incubating or culturing the tissue and the cells in an environment suitable for the growth of the cells and the tissue for a time sufficient to obtain measurements with respect to one or more parameters, and E) measuring some of those one or more parameters to obtain data regarding those behaviors.

[0015] Methods of the present disclosure are further adapted such that, preferably, the implantation of the sample, or portions thereof, is effected with minimal disruption to the cells or the tissue. For example, in a typical procedure according to some preferred embodiments of the disclosure, a sample quantity of a tissue is removed from a patient by way of a biopsy or surgery. The structural integrity of that tissue sample is then maintained to the maximum extent possible before its implantation. In this regard, it is noteworthy that non-disruption of the sample means, as examples, that the sample is not homogenized, it is not chemically disrupted by strong acids, or by enzymes such as trypsin, nor is it comminuted, crushed or subjected to high concentrations of strongly basic compounds. In sharp contrast, a sample according to the present disclosure is cut, torn or chopped into small pieces, such as chunks approximating cubes of 1.0 – 3.0 mm on an edge. While not intending to be bound by any underlying mechanism, the present inventors theorize that, by minimizing the disruption to the sample tissue and cells, their respective behaviors in relation to a physiological matrix of the disclosure remains similar to their corresponding behaviors in vivo.

[0016] In this same vein, it is preferable that implantation of the samples during the methods of the present disclosure take place as soon after the sample is removed from the animal as is reasonably possible. Thus, a removed tissue sample is preferably immediately cooled by placing it in proximity to ice, for example, and implantation is most preferably accomplished within one or a few hours after removal. Although implantation is most preferably accomplished within an hour or so of sample removal, the present methods provide for later implantation when circumstances dictate as much, such as when the sample must be transported some distance to a laboratory for preparation. In any event, implantation should most preferably take place within four hours of removal of the sample tissue, or more preferably within 24 hours of removal, and less preferably within 48 hours of removal of the tissue from the subject animal. As one of skill in the oncological arts will appreciate, the effects of the delay in implantation and evaluation of a tissue sample may depend upon many factors, including the type of tissue, its oncological status, and the conditions under which it has been stored. Thus, it may be possible to delay implantation for many hours or many days.

[0017] As another advantage of the present disclosure, its means and methods provide data for physicians and other evaluators to make determinations or estimates regarding the oncological status of a tissue and its cells. Thus, from the data obtained, a

determination can be made regarding whether the tissue and cells are abnormal, pre-cancerous or cancerous. As a further advantage, the present means and methods permit the testing of many types of therapies, including pharmaceutical compounds and non-chemical therapies. The present means and methods can therefore be used to provide data useful for tailoring specific individual therapeutic regimens directed toward the precise tissue being evaluated. To this end, some preferred embodiments of the present

disclosure further comprise the step of transmitting the data to at least one evaluator. In the context of the present disclosure, an evaluator is any person, group of people, or network of people, or any machine, computer or device adapted and arranged for evaluating the data garnered through use of the present means and methods. Evaluators include, but are not limited to the group comprising technicians, technicians, physicians, physician's staff members, physician's assistants, hospital employees, clinic employees, the patient, technologists, technical assistants, laboratory assistants, oncological analysts, nurses, nurses' assistants, nurse practitioners, computers, computer-aided devices, computer-facilitated devices, and human or computerized agents for any of the foregoing.

[0018] In accordance with still further embodiments of the present disclosure, the removed sample quantity of tissue is divided into a plurality of portions, and each of the portions is subjected to the some or all steps of the present disclosure. Thus, tests and evaluations of the tissue and cells can be performed in multiples, and one or more therapies can be tested concurrently in relation to the specific sample if desired.

[0019] Moreover, the measurements taken with respect to each portion of a sample are taken over time, either at periodic intervals, or at random intervals. In some preferred embodiments therefore, Step E is performed more than once, or a plurality of times with respect to the separate portions of the sample. Preferably, these measurements are effected during the culturing or incubating of the tissue and cells as in Step D above.

[0020] Advantageously, means and methods of the present disclosure can be used on virtually any type of animal, including human medical patients, and can be used also to evaluate tissue from other animals. Other animals include, but are not limited to non-human primates, equines such as horses and donkeys, bovines such as cows and deer, canines such as dogs and wolves, felines such as lions and domestic house cats, murines such as mice and moles, porcines such as pigs and peccaries, avians such as birds and penguins, amphibians such as salamanders and turtles, and reptilians such as snakes and alligators. The animals mentioned herein comprise an exemplary listing, and not an exclusive one.

[0021] Methods of the present disclosure also include where the measurements taken, as in Step E, are performed more than once with respect to each of the separate portions of the sample, and wherein the measuring is effected during the culturing or incubating of Step D. A significant embodiment of the present disclosure concerns the physical relationship between the tissue sample portion and the three-dimensional matrix in which it is implanted. Preferably, the tissue portion is disposed either partially submerged in the matrix, or completely submerged therein. It is important that the tissue be in intimate contact with the matrix. Thus, the physiological matrix replicates to some extent the three-dimensional environment in which most tissues function in vivo. Tissue samples, or portions thereof, may be implanted in any method, manner or way, which yields the desired results

[0022] As an aid to implantation, the matrices of the present disclosure, which are typically gels or other permeable solids or semi-solids, can be provided in shapes which facilitate the implantation of one or more types of tissues and cells, and which facilitate the provision of nutrition to the cells and tissue. Thus, any type of depression such as a hollow, cavity, slit, chamber or slot can be provided within the matrix itself, and can be adapted and arranged to receive the tissue or cells for implantation, or can be adapted and arranged to hold nutrition for the cells and tissue. For example, a gel matrix according to the present disclosure may comprise a hollow, cavity, slit, chamber or slot of an appropriate volume and disposition to receive an aliquot, such as a .2 ml. aliquot of 10X media, as nutrition for the implanted cells and tissue. Such depressions can also serve as receptacles or reservoirs for drugs to be tested.

[0023] In some preferred embodiments of methods of the present disclosure, the tissue or cells are implanted in the matrix as the matrix is being formed into a gel or other permeable solid or semi-solid. In these embodiments, the tissue and cells are mixed along with the components of the matrix and are thus partially or completely submerged within the matrix by the time it gelates or hardens into a semi-solid or solid. In those preferred embodiments where the matrix is already formed, the tissue or cells can be implanted in the matrix by physical insertion after it has formed into a gel, solid or semi-solid.

[0024] It is important to note that solutions for forming matrices of the present disclosure should be essentially free of bicarbonate. By maintaining this bicarbonate-free condition, the gels or semi-solids of the present disclosure advantageously form at room temperatures and are thus capable of being mixed and formed in a manner that is convenient, user friendly, and adaptable to many laboratory and non-laboratory environments.

[0025] In accordance with other embodiments of the present disclosure, sufficient nutrition is provided to the cells and tissue to be evaluated or tested, either as a component of the matrix as it is being formed, or as nutrition added after the matrix is formed, or both. The nutrition must be sufficient to grow the cells and tissue for a sufficient length of time to obtain the desired results. In some embodiments, the sufficient or additional nutrition preferably comprises one or more fluids, wherein the one or more fluids are selected from the group comprising tissue culture medias, concentrated tissue culture medias, and any culture or nutritional medias which are suitable for the tissue sample. The means and methods of the present disclosure thus include wherein the concentrated medias are one or more selected from the group comprising 10X medias, and any other concentrated medias.

[0026] In another embodiment of the present disclosure, the three-dimensional physiological matrix comprises the sufficient nutrition as a component of the matrix itself as it is being mixed and formed or allowed to gel. As a further embodiment of the present disclosure, a step of providing at least one source of additional nutrition to the tissue and the cells is provided. Thus, the tissues and cells may avail themselves of one or both of the sufficient nutrition and of the additional nutrition to the extent necessary so that the parameters can be measured. In accordance with similar embodiments of the present disclosure, the at least one source of additional nutrition may comprise one or more of tissue culture medias, concentrated tissue culture medias, and any culture or nutritional medias which are suitable for the tissue sample. As a further embodiment of the present disclosure, the at least one additional source of nutrition may be supplemented with one or more components selected from the group comprising sera, proteins, sugars, salts, lipids.

[0027] A further method of the present disclosure wherein the measuring of Step E is performed at periodic intervals. Preferably the measuring step is performed at periodic intervals such as one or more intervals selected from the group comprising every six hours, every 12 hours, every 18 hours, every 24 hours, every 36 hours, every 48 hours, every 72 hours and every 96 hours. Moreover other intervals are one or more selected from the group comprising every day, every second day, every third day, every fourth day, and every fifth day.

[0028] As another advantage, the data obtained by the measurements of the present disclosure is recorded in a fixed media. Such fixed media can be any type so long as it is capable of holding the obtained data in such form as to be useable later. For example, paper, voice recordings, video recordings, photographs, photomicrographs, digital recordings, any

fixed digital data means. According to the present disclosure one or more of a human observer, a still camera, a video camera, an automated still camera, an automated video camera, an infrared camera, and an automated infrared camera can be used to obtain the desired data.

[0029] Preferably the culturing of tissues and cells according to the present disclosure occurs in a period of time sufficient to obtain the desired parameters and values. For example, the periods of time during which the culturing of tissues and cells is effected includes those of at least 10 hours, at least 36 hours, at least 72 hours and at least 125 hours, or even longer depending on the conditions of the tissue and how the method is being conducted

[0030] In accordance with other embodiments of the present disclosure, the sufficient nutrition is provided to the tissues and cells by placing the sufficient nutrition in contact with the matrix after the sample has been placed in the matrix and the matrix has equilibrated to room temperature, and to a semi-solid or gel. Preferably, the room temperature is in the range of from 10 to 30 degree Celsius. In accordance with other embodiments, the sufficient nutrition may be provided to the tissues and the cells by placing the it in contact with the matrix after the sample has been placed in the matrix, and the matrix has equilibrated to a gel at room temperature. In other embodiments, the sufficient nutrition may be provided to the tissues and the cells by continuous or intermittent perfusion or flow over, around, or through the matrix. In yet another embodiment, the three dimensional quality of the matrix of the present disclosure can be formed itself to comprise the sufficient nutrition. The environment for incubating or culturing the cells and the tissue preferably includes a temperature range of from 30 to 40 degrees Celsius, a carbon dioxide tension of from 2 to 13%, and a relative humidity of from 50% to 100%.

[0031] In accordance with other embodiments, methods of the present disclosure can be conducted wherein the sample quantity is preferably divided into portions, and each of these portions is subjected concurrently and separately to the method, wherein the matrix comprises a gel, and wherein the gel is suitable for measuring the one or more parameters to obtain the desired values. Thus, multiple equivalent matrices may be formed in multiple containers, such as multiple containers comprising a multi-well apparatus, such that multiple portions of the sample can be evaluated concurrently. Thus, the present disclosure includes where a particular tissue can be evaluated at multiple times, with respect to multiple portions of the sample, and with respect to multiple therapies.

[0032] Advantageously, the present disclosure can be used to measure or obtain data with respect to one or more parameters regarding the behavior of the cells and tissue. Those parameters include one or more of the distance which individual cells migrate from the tissue sample, the average distance of migration of a group or population of the cells from the tissue sample, the distance which individual cells migrate from the tissue sample with respect to time, the average distance of migration of a group or population of the cells from the tissue sample with respect to time, the velocity of migration of one or more designated individual the cells per selected time period, the velocity of migration of a group or population of designated the cells per selected time period, the number of migrational cells per unit area of a microscopic visual field, the speed of proliferation of the cells, the speed of unidirectional migration of the cells, the speed of bi-directional migration of the cells, the speed of tri-directional migration of the cells, the frequency of directional change of the migrating cells, the number of directional changes per unit time of the migrating cells, the rate of mitosis of the migrating cells, the number of cells migrating per unit time, the number of cells in a unit area of a portion of the gel, the change in the number of cells in a unit area of a portion of the gel with respect to time, the number of cells in a unit volume of a portion of the gel with respect to time, and the change in the number of cells in a unit volume of a portion of the gel, the proportion of cells which change migrational direction during the test period, the speed of migrating cells in a particular visual field, the density of migrating cells per unit area of a visual field, the migrational distance of individual migrating cells per unit area of a visual field, the average migrational distance of a group or population of migrating cells per unit area of a visual field, osmotic pressure, ionic strength, the change in pH with respect to time, the change in pH with respect to the amount of cell migration, oxygen consumption, glucose consumption, and the number of migrating cells in a particular direction per unit area of a visual field.

[0033] In accordance with other advantageous embodiments of the present disclosure the three-dimensional physiological matrices for use with the invention can be adapted and arranged to have whatever pH value or range necessary to culture and evaluate the specific target tissues and cells. Preferably, the pH range for testing most tissues is from 5.0 to 8.0, and, more preferably, from 7.0 to 7.5. Thus, the physiological matrix preferably has a pH which is adapted to a range suitable for one or more of a particular tissue, a particular cell type or types, or to a particular tumor.

- [0034] Preferably, a three dimensional matrix of the present disclosure comprises at least one natural or synthetic fiber, for example, one or more fibers selected from the group including type I collagens, type II collagens, type III collagens, type IV collagens, fibrin, fibrinogen, extracellular matrix proteins derived from one or more animals, laminin, fibronectin, anti-laminin, and other natural or synthetic fibers or cables suitable to the formation of a matrix mesh or network capable of supporting cell support, movement and growth. In some preferred embodiments a physiological matrix according to the present disclosure preferably comprises a gel, the gel being suitable for measuring at least one or more of the parameters necessary for evaluating the tissue, the cells, possible therapies, and is also adapted and arranged for measuring the efficacy of one or multiple therapeutic compounds.
- [0035] The means and methods of the present disclosure are especially adapted and arranged to test the efficacy of virtually any therapy regimen, including pharmacologic therapeutics. In one context therefore, a physiological matrix of the present disclosure is adapted and arranged for measuring the efficacy of one or more therapeutic compounds, such as known and unknown single compounds, and known and unknown therapeutic cocktails. As examples, and not as an exclusive listing, one or more therapeutic compounds for use or evaluation in the context of the present disclosure may be selected from the group comprising anti-tumor agents, DNA damaging agents such as alkylating agents, antibiotics which affect nucleic acids, platinum compounds, anti-mitotics, cell cycle stimulators, anti-metastatic agents, anti-metabolites, camptothecin derivatives, hormone therapies, biological response modifiers, interferon, anti-invasives, anti-invasion agents, anti-migration agents, anti-angiogenesis agents, and apoptotic agents, radiosensitizers, radiation, and any known, theoretical or experimental therapeutics directed against cell growth, cell invasion or cell viability, pro-tumor agents, pro-mitotic agents, pro-metastatic agents, pro-invasion agents, pro-migration agents, pro-angiogenesis agents, and anti-apoptotic agents.
- [0036] More specially, the three-dimensional physiological matrices of the disclosure may be adapted and arranged for measuring the efficacy of one or more therapeutic compounds, such as those selected from the group comprising Temozolomide (TMZ), Irinotecan, Procarbazine, Methotrexate, Carboplatin, Adriamycin Cisplatin, Vincristine, Paclitaxel, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), carmustine (BCNU), Cyclophosphamide, Docetaxel, fluorouracil (5FU), Cytarabine, doxorubicin,

bleomycin, topotecan, tamoxifen (TMX), and imatinib mesylate (Gleevec) and all other therapeutics shown to be appropriate for treatment.

[0037] As additional examples, a physiological matrix of the present disclosure is adaptable for measuring one or a cocktail of therapeutic compounds comprising one or more, or at least two compounds selected from one or more of CAF (Cyclophosphamide/Adriamycin/ Fluorouracil); CMF (Cyclophosphamide/ Methotrexate Fluorouracil); CMFVP (Cyclophosphamide/ Methotrexate/Fluorouracil/ Vincristine/ Prednisone); PCV (Procarbazine/CCNU/ Vincristine); ICARBO-E (Ifosfamide/ Carboplatin/Etoposide); TAP (Taxol/Adriamycin/ Cisplatin); EMA-CO (Etoposide/ Methotrexate/Actinomycin/ Cyclophosphamide/ Vincristine); VBP (Vinblastine/ Bleomycin/Platinol (Cisplatin)); BPD-T: (Bcnu/Platinol/ Dacacarbazine/ Tamoxifen); and T-10 (Methotrexate/ Bleomycin/Cyclophosphamide/ Dactinomycin/Adriamycin), Dactinomycin/Adriamycin) and any other actual or theoretical therapeutics which might be appropriate for treatment.

[0038] As yet another advantage, the means and methods of the present disclosure may be adapted and arranged to test the efficacy of non-chemical therapies. As examples, and not to be taken as an exclusive listing, non-chemical therapies subject to being evaluated or tested in the context of the present disclosure include one or more selected from the group comprising radiation therapies, brachiotherapies, herbal therapies, naturopathic therapies, experimental therapeutic compounds, and new therapeutic compounds.

[0039] In additional embodiments, the method may further comprise the step of determining the oncological status of the cells of the removed tissue from the data thus obtained. Preferably, the methods may further comprise the step of recording the data in some fixed media so that it can be analyzed, communicated to others, and used as a record of the procedures and their results.

[0040] In accordance with other objects of the present disclosure, methods for evaluating the efficacy of one or more therapeutic compounds with respect to one or more tissues comprising cells from an animal are provided. Methods of the present disclosure are particularly relevant with respect to tissue and cells which are suspected of being abnormal, cancerous or pre-cancerous. Indeed, the present means and methods are especially adaptable to testing or evaluating one or multiple therapeutic compounds with respect to specific tissue and cells. As a result of this ability to evaluate potential therapies, such as therapeutic compounds, with respect to an exact tissue, time, effort

and expense are saved in terms of the determination of an appropriate therapeutic regimen.

[0041] In one preferred embodiment, the present methods for evaluating one or multiple therapeutic compounds comprises the steps of A) obtaining a sample of the suspected tissue from the animal, wherein the tissue comprises one or more types of cells, B) implanting a portion of the sample of the suspected tissue at least partially within a three-dimensional physiological matrix, the matrix being adapted and arranged for measuring one or more parameters of the behavior of the tissue and the cells, C) providing sufficient nutrition to the cells and the tissue so that the parameters can be measured, D) providing at least one of the one or more therapeutic compounds to the cells and the tissue, E) incubating or culturing the tissue and the cells with the one or more therapeutic compounds in an environment suitable for the growth of the cells and the tissue, and for a time sufficient to obtain measurements with respect to the one or more parameters, and F) measuring one or more of the parameters to obtain data regarding the behaviors with respect to the one or more therapeutic compounds.

[0042] Advantageously, the methods may further comprise the step of, from the data thus obtained, determining the efficacy of the one or more therapeutic compounds with respect to the tissue and the cells. Preferably, methods of the present disclosure may further comprise the step of recording the data in some fixed form, and may further comprise the step of transmitting the data to at least one evaluator. Evaluators and the recordation of data obtained by the present means and methods are also discussed herein.

[0043] The present disclosure is also especially adapted for circumstances wherein the sample quantity is divided into portions, and each of the portions is subjected concurrently and separately to the method, wherein the method includes the use of one or more controls adapted for evaluating the one or more therapeutic compounds. Thus, the present methods can include the use of one or more controls adapted for evaluating the one or more therapeutic compounds with respect to the particular tissue, and with respect to specific conditions created by the test or testing lab or clinic. Methods of the present disclosure also include where Step D is performed during Step E, and wherein the measuring of Step F is performed more than once with respect to each of the separate portions of the sample, and wherein the measuring is effected during the culturing or incubating of the tissue and cells. Preferably, the tissue portion is disposed either partially submerged in the matrix, or completely submerged therein.

- [0044] It is important that the tissue be in intimate contact with the matrix. Thus, the physiological matrix replicates to some extent the three-dimensional environment in which most tissues function in vivo. Tissue samples, or portions thereof, may be implanted in any method, manner or way, which yields the desired results. Means and methods of the present disclosure can be used also where the implanted tissue is obtained from one or more of a liquid tissue, a cell suspension, cells or groups of cells which have been palletized, and wherein the tissue or cells are implanted in the matrix after the matrix has formed into a gel or other permeable solid or semi-solid.
- [0045] Means and methods of the present disclosure can be used with virtually any animal tissue as described herein. Moreover, it is important to note that matrices of the disclosure should be essentially free of bicarbonate when the matrix is being formed into the gel, semi-solid or permeable solid. As an additional advantage, methods of the present disclosure can be practiced with respect to one or more therapeutic compounds, for example where a single compound is tested and the method is performed with at least one set of controls. As one skill in the art of testing oncological remedies will appreciate, any number of controls, therapeutic substances, therapeutic methods, experimental compounds, or other cancer treatments such as radiation, radiotherapy or any other actual, theoretical or experimental therapy or method can be tested with the present disclosure in in vitro conditions.
- [0046] Moreover, therapeutic compounds can be provided to the tissues and cells as a component of the sufficient nutrition. More therapeutic compounds are provided to the cells and the tissue as a component of the physiological matrix. As a component after the sufficient nutrition is provided to the cells and tissues or wherein the one or more therapeutic compounds include two compounds and the method is performed with at least one set of controls. It is similarly possible to use the present disclosure and methods to test three or more therapeutic compounds preferably with respect to at least one set of controls. Methods of physiological matrix is adapted and arranged for measuring the efficacy of one or more therapeutic compounds, and wherein the one or more therapeutic compounds are selected from the group comprising DNA damaging agents such as alkylating agents, antibiotics which affect nucleic acids, platinum compounds, anti-mitotics, cell cycle stimulators, anti-metastatics, anti-metabolites, camptothecin derivatives, hormone therapies, biological response modifiers, interferon, anti-invasives, radiosensitizers, radiation, and any established, theoretical or experimental therapeutics directed against cell growth or cell invasion.

- [0047] The present means and methods with respect to a three-dimensional physiological matrix of the disclosure are adapted and arranged for measuring the efficacy of one or more therapeutic compounds,
- [0048] More specially, a physiological matrix of the present disclosure is adapted and arranged for measuring the efficacy of one or more therapeutic compounds comprises at least two therapeutic compounds. And where at least two therapeutic compounds are selected from one or more of
- [0049] It is again necessary to mention that the physiological matrices according to the present disclosure should be essentially free of bicarbonate, for example wherein the matrix is formed from components that are essentially free of bicarbonate, and the final gel form of the matrix is thus essentially bicarbonate free.
- [0050] As another distinct aspect of the present methods one or more parameters can be obtained regarding the behaviors of one or more therapeutic compounds, and the behaviors of the cells themselves. Parameters thus measured yield data. In turn, such data can be used to evaluate the performance of the cells, or the performance of one or more therapeutics, or the performance of one or more other types of therapies with respect to the tissues and cells tested. Preferably, the measuring step E is performed at periodic intervals, and wherein the periodic intervals are appropriate to one or more of the cells, the tissue and the therapy or therapies being tested or evaluated.
- [0051] Preferably the measuring of Step E is performed at periodic intervals for a period of time of between one hour and 15 days. More preferably, the periodic intervals are one or more selected from the group comprising every six hours, every 12 hours, every 18 hours, every 24 hours, every 36 hours, every 48 hours, every 72 hours and every 96 hours. Moreover other intervals such as every day, every second day and every third day, every fourth day, every fifth, day every sixth day, every seventh day, every eighth day, and every ninth day. Methods of the present disclosure wherein the measuring of Step E is performed at non-periodic intervals, and wherein the non-periodic intervals are appropriate to one or more of the cells, the tissue and the therapy or therapies being tested or evaluated.
- [0052] As another advantage, the data obtained by the measurements of the present disclosure is recorded in a fixed communications media. Such fixed media can be any type so long as it is capable of holding the obtained data in such form as to be useable later. For example paper, voice recordings, video recordings, photographs, photomicrographs, digital recordings, any fixed digital data means, any computer network-facilitated

means, infrared recording, ultrasound recordings, and magnetic resonance recordings.

According to the present disclosure one or more of a human observer, a still camera, a video camera, an automated still camera, an automated video camera, an infrared camera, and an automated infrared camera.

[0053] Preferably the culturing and/or incubating of tissues and cells according to the present disclosure occurs for a period of time sufficient to obtain data regarding the desired parameters and values. For most tissue and cells to be tested, that period of time is between one hour and 15 days. More specifically, the culturing or incubating of the tissue and the cells typically occurs for a period of time of at least 1 hour, or at least 10 hours, or at least 24 hours, or at least 36 hours or at least 72 hours, or at least 125 hours, or at least 175 hours, or at least 200 hours, or at least 250 hours or at least 300 hours.

[0054] In some preferred embodiments, the environment for incubating or culturing the cells and the tissue includes a temperature range of from 26 to 43 degrees Celsius, a carbon dioxide tension of from 2.0% to 13.0%, and a humidity range of from 50-100 %.

[0055] In accordance with other embodiments of the present disclosure, the sufficient nutrition comprises one or more fluids, and wherein the one or more fluids are selected from the group comprising tissue culture medias, concentrated tissue culture medias, and any culture or nutritional medias which are suitable for the tissue sample. Preferably the concentrated medias are one or more selected from the group comprising 10X medias, and any other concentrated medias. Preferably, the sufficient nutrition is provided to the tissues and cells by placing the sufficient nutrition in contact with the matrix after the sample has been placed in the matrix.

[0056] The method of the present disclosure further comprises the step of J) providing at least one source of additional nutrition to the tissue and the cells so that the parameters can be measured. Wherein at least one source of additional nutrition comprises one or more of tissue culture medias, concentrated tissue culture medias, and any culture or nutritional medias which are suitable for the tissue sample and wherein least one additional source of nutrition is supplemented with one or more selected from the group comprising sera, proteins, sugars, salts, lipids. Preferably the sufficient nutrition is provided to tissues and cells by placing the sufficient nutrition in contact with the matrix after the sample has been placed in the matrix and the matrix has equilibrated to room temperature, wherein the room temperature is in the range of from 10 to 30 degrees Celsius.

[0057] Advantageously, the present disclosure can be used to obtain data regarding one or multiple parameters of the behaviors of the tissue and cells being evaluated or tested as

discussed herein. Significant among those parameters are the distance which individual cells migrate from the margins of the tissue sample, the average distance of migration of a group or population of the cells from the margins of the tissue sample, the distance which individual cells migrate from the tissue sample with respect to time, and the number of migrational cells per unit area of a microscopic visual field; that is, cell density in a portion of the gel.

[0058] As an additional advantage, methods of the present disclosure may include those for evaluating the efficacy of one or more therapies with respect to at least one tissue from an animal, the tissue comprising cells, and the tissue being suspected of being abnormal, pre-cancerous or cancerous, the method comprising the steps of A) obtaining a sample of the tissue from the animal, wherein the tissue comprises one or more types of cells, B) implanting the sample at least partially within a three-dimensional physiological matrix, the matrix being adapted and arranged for measuring one or more parameters of the behavior of the cells of the tissue or cells, C) providing sufficient nutrition to the cells of the tissue of the portions so that the parameters can be measured, D) subjecting the cells and the tissue to at least one of the one or more therapies, E) incubating or culturing the tissue and the cells of the portions in an environment suitable for the growth of the cells and the tissue for a time sufficient to obtain measurements with respect to the one or more parameters for each of the portions, and with the one or more therapeutic compounds, F) measuring, with respect to each of the portions, one or more of the parameters to obtain data regarding the behaviors with respect to the one or more therapies.

[0059] In accordance with these and other objects of the present disclosure, a three-dimensional physiological matrix suitable for culturing at least one tissue from an animal, the tissue comprising cells, is provided. In one embodiment, the tissue or cells of the sample are suspected of being abnormal, cancerous or pre-cancerous. In another, the physiological matrix comprises A), at least one natural or synthetic fiber means, wherein the fiber means is adapted and arranged such that the cells, if abnormal, cancerous or pre-cancerous, are enabled to grow away from the sample into the matrix when the sample is implanted at least partially in the matrix, B), a sufficient amount of at least one additive comprising sufficient hydroxyl or hydrogen groups to bring the effective pH range of the matrix into an acceptable range, and C), sufficient nutrition to sustain the cells and the tissue in the matrix. It is important to note that the matrices of the

invention and the sufficient nutrition should be essentially free of bicarbonate when they are being formed.

- [0060] The three-dimensional physiological matrix is mixed from components and formed into a solid, a semi-solid, or a gel, and the matrix is adapted and arranged to permit migration of the cells away from the margins of the tissue and into the matrix. Moreover, the matrix is formulated, adapted and arranged to accept the sample as soon as the solid or gel is formed, or during its formation.
- [0061] Preferably, a three-dimensional physiological matrices of the present disclosure comprise at least one natural or synthetic fiber, for example, one or more selected from the group comprising type I collagens, type II collagens, type III collagens, type IV collagens, fibrin, fibrinogen, laminin, anti-laminin, positively charged poly(L-lysine) amino acid chains, positively charged poly(D-lysine) amino acid chains, and extracellular matrix proteins derived from one or more animals. In one preferred embodiment, the matrix comprises at least 90% Type I collagen. In another preferred embodiment of a matrix according to the present disclosure, the gel comprises at least 95% Type I collagen.
- [0062] The pH of a three-dimensional physiological matrix of the present disclosure is brought into an acceptable range by means of one or more additives preferably selected from the group comprising NaOH, KOH and other strongly basic compounds. A matrix of the present disclosure may further comprise additional nutrition to be added after the formation of the matrix into a solid, semi-solid or gel.
- [0063] In accordance with other embodiments of the present disclosure, the sufficient nutrition comprises one or more fluids, preferably selected from the group comprising tissue culture media, concentrated tissue culture media or any culture or nutritional media which are suitable for the tissue sample to be evaluated. Suitable concentrated medias include one or more selected from the group comprising 10X media, and other concentrated media. Preferably, the sufficient nutrition is provided to the tissues and cells by placing the sufficient nutrition in contact with the matrix after the sample has been placed in the matrix and wherein one additional source of nutrition is supplemented with one or more selected from the group comprising sera, proteins, sugars, salts, lipids, and the matrix has equilibrated to room temperature, wherein room temperature is in the range of from 10 to 30 degrees Celsius.
- [0064] In accordance with other advantageous embodiments, a three-dimensional physiological matrix for use in the context of the present disclosure can be adapted or arranged to have whatever pH value or pH ranges necessary to culture and evaluate the specific tissues

and cells. Preferably, the pH range for testing most tissues is from 5.0 to 8.3, more preferably 6.4 to 7.9 and even more preferably from 7.4 to 7.6. Preferably, the tissue to be evaluated or tested is implanted or disposed completely submerged within the three-dimensional matrix.

[0065] In accordance with other embodiments of the three-dimensional physiological nature of the present disclosure, a matrix is preferably adapted and arranged to be in sufficient proximity to at least one additional source of nutrition, wherein the matrix is permeable to the additional source of nutrition to the extent necessary that the sample tissue and cells receive nutrition sufficient to enable them to grow into the matrix to the extent necessary to measure the one or more parameters. Moreover, the at least one additional source of nutrition may preferably include one or more additives or supplements selected from the group comprising serums, proteins, sugars, salts and lipids or any culture media that is suitable for the cell or tissue type. Preferably, the density of the fiber in a formed matrix of the invention is between 1.3 and 3.3 mgs/ml, more preferably from 2.0 to 2.8 mgs/ml, and even more preferably between 2.3 and 2.5 mgs/ml.

[0066] Advantageously, the present disclosure can be adapted and arranged to be suitable for measuring at least one parameter of the behavior of cells from the tissue, and at least one parameter relating to the suspected cancerous or abnormal status of the cells or the tissue. These parameters are listed hereinbefore.

[0067] In a significant embodiment, a three-dimensional physiological matrix of the present disclosure includes at least one natural or synthetic fiber selected from the group comprising Type I collagen, wherein the Type I collagen comprises at least 70% of the total fiber of the formed matrix, or at least 80% of the total fiber, at least 90% of the total fiber and at least 95% of the total fiber. In one preferred embodiment, a three-dimensional physiological matrix of the present disclosure comprises at least one natural or synthetic fiber means of primarily Type I collagen, and at least one additive sufficient to bring the pH of the matrix into an acceptable range, such as a sufficient amount of 0.1 N NaOH, and wherein the sufficient nutrition is a bicarbonate-free 10X media.

[0068] In another preferred embodiment, a three-dimensional physiological matrix of the present invention comprises eight parts by volume of the natural or synthetic fiber, the at least one additive comprises one part by volume of the 0.1 N NaOH, and the sufficient nutrition comprises one part by volume of the bicarbonate-free 10X media.

[0069] In another embodiment, a physiological matrix according to the present disclosure may comprise at least one first hollow, cavity, slit or chamber, and the first hollow, cavity slit or chamber may be adapted and arranged to receive one or more tissues samples, wherein the one or more tissues samples are in the form of one or more of a solid, a liquid, a suspension, and combinations thereof. Moreover, a three-dimensional matrix of the present disclosure may comprises at least one second hollow cavity, slit or chamber, wherein each of the second hollows, cavities, slits or chambers is adapted and arranged to receive at least a portion of the additional source of nutrition.

[0070] Preferably, the three-dimensional matrix of the disclosed matrix comprises a gel, the gel being suitable for measuring at least one of the one or more parameters and of producing resulting data. Moreover, the physiological matrices of the present disclosure are adapted and arranged for measuring the efficacy of one or a plurality of therapeutic compounds, many of which are exemplified herein. Moreover, means or methods of the present disclosure can be adapted and arranged to test the efficacy of non-synthetic chemical therapies, wherein the non-synthetic chemical therapies are one or more selected from the group comprising radiation therapies, brachiotherapies, herbal therapies, naturopathic therapies, experimental therapeutic compounds, and new therapeutic compounds, hyperthermal therapies, hyperbaric therapies, hypobaric and photosensitizing therapies.

[0071] An additional benefit of the present disclosure pertains to its amenability to kit form. Thus, in some embodiments, the present disclosure may include a kit for testing the efficacy of therapeutic compounds on an expedited basis, the kit comprising a means for collecting at least one tissue comprising cells from an animal, the tissue being suspected of being abnormal, cancerous or pre-cancerous, at least one three-dimensional physiological matrix for culturing the tissue sample in three dimensions for a sufficient length of time to obtain measurements of at least one parameter of the behavior of the cells and the tissue, a means for measuring the parameters to determine a set of possible therapeutic compounds which might be efficacious for treating the tumor or abnormal tissue, and a means for testing the therapeutic effect of the determined therapeutic compounds.

[0072] Preferably, the behavior of cells at multiple sites, for example, at least 2 sites or at least 3 sites, or at least 4 sites, or at least 5 sites is measured to obtain data regarding the behavior of the tissue or cells. In some preferred embodiments, readings made using microscopy and video-microscopy include the actions or steps of measuring directly the

migratory distance, speed, cell density and direction of movement of the cells or tissue with respect to the matrix upon which they are placed or cultured.

[0073] In other preferred embodiments of the present disclosure, the suspect tissue and matrix are then processed and treated so that they can be snap frozen, paraffin embedded, sectioned, and then assessed with respect to one or more parameters, including, but not limited to, mitotic index/cell division, cell growth, necrosis, apoptosis, anoikis, adhesion, cellular signaling and invasion-related protein expression. Any number of protein and genetic parameters can be analyzed with respect to their usefulness in determining the oncologic state of the cells or tissues.

[0074] In accordance with certain other embodiments of the present disclosure, the matrix may be of any configuration or material which permits the evaluation of the effect of therapeutic compounds on the cells or tissues. In some embodiments of the present disclosure, the matrix preferably comprises a collagen gel, the gel being suitable for measuring the one or more parameters. Matrices could include any protein or glycoprotein or sugar components of extracellular matrix, for example, including but not limited to, fibronectin, laminin, hyaluronin, growth factors and integrins.

[0075] In general, in one set of preferred embodiments, the typical matrix element of the disclosure comprises a gel. In one set of especially preferred embodiments, a matrix of the disclosure comprises protein in its native, non-denatured state, in the amount of 1.0 to 2.1 mg/ml. One preferred protein is, for example, Type I Collagen. Other proteins are amenable to use or adaptation as components of matrices of the invention. In some embodiments, a matrix of the present disclosure possesses a final pH preferably in the range of from 6.0 to 8.5, more preferably in the range of from 6.5 to 8.0, and most preferably in the pH range of from 6.8 to 7.5

[0076] In other embodiments, a matrix of the present disclosure is incubated and used in an air atmosphere having carbon dioxide preferably in the range of 1.0 to 18.0%, more preferably in the range of from 2.0 to 14.0%, and most preferably in the range of from 4.0 to 9.0%. A carbon dioxide incubator is useful for incubation processes according to the present disclosure. In accordance with other embodiments of the present disclosure, the parameters of matrix composition, carbon dioxide tension, and other conditions vary according to tumor type. For example, for most categories of tumors, temperatures for incubation of the matrices combined with tissue or cells are generally kept within approximate physiological ranges, preferably of from 30 – 44 degrees Celsius, more preferably of from 33 – 41 degrees Celsius, and most preferably of from 35 – 39 degrees Celsius.

- [0077] In another advantageous embodiment of the present disclosure, a matrix may comprise one or more constituents provided to adapt the matrix for use with a particular tumor, or a particular class of tumors. Possible constituents to a matrix according to the present disclosure, for example a gel matrix, may include growth factors such as VEGF or HGF, which tends to alter the invasion patterns of the tissue cells into the matrix and enhances cell growth. Other possible constituents include structural proteins, for instance, extracellular matrix proteins like laminin, fibronectin, and the various collagens, for example Collagen IV.
- [0078] Immunoglobulins may also be included or added to a matrix of the present disclosure in order to stabilize it in various respects. For instance, anti-laminin may be included in the matrix. As additional means and methods for adapting matrices of the disclosure to uses with respect to specific tumors or classes of tumors, lipids, such as human or bovine brain extract, solids like human dura, and plant extracts such as phytostimulants and inhibitors may also be included in a matrix of the invention in order to vary its characteristics and adapt it to specific use. In some embodiments, other living cells may be included. Exemplary cells include melanoma cells, fibroblasts and endothelial cells
- [0079] Matrices of the present disclosure may include inert additives also. As examples, glass or plastic shapes, such as cylinders may be provided within tissue culture wells to act as structural forms for the gel so that the matrix gel can be molded to create a tiny well or indentation useful for adding cell suspensions such as leukemia or lymphoma cells to the matrix in a pre-determined area in the matrix gels.
- [0080] In accordance with preferred embodiments of the present disclosure, the one or more parameters to be measured comprise one or more of: the speed of proliferation of the cells, the speed of unidirectional migration of the cells, the speed of bi-directional migration of the cells, the speed of tri-directional migration of the cells, the rate of mitosis of the cells, the rate of change of migrational direction of the cells, the proportion of cells which change migrational direction during the test period, the speed of migrating cells in a particular visual field, the density of migrating cells per microscopic visual field, the migrational distance of individual migrating cells per microscopic visual field, the average migration-al distance of a group or population of migrating cells per microscopic visual field, and the number of migrating cells per microscopic visual field direction.

[0081] With respect to the invention, the "Test Period" is that period of time during which the one or more tissues or cells are cultured and tested with respect to their oncological behaviors, and the "Evaluational Period" is that period of time during which the one or more therapeutic compounds are evaluated with respect to efficacy in treating the specific tissue samples or cells, and other parameters.

[0082] In accordance with the many advantageous embodiments of the methods of the present disclosure, the one or more therapeutic compounds or treatments are selected from the group comprising, but not limited to, DNA damaging agents, anti-mitotics, cell cycle stimulators, anti-metastatics, anti-invasives, radiosensitizers, radiation, and any established, theoretical or experimental therapeutics against cell growth and cell invasion.

[0083] Methods of the present disclosure include the evaluation of one or more therapeutic compounds, individually, combined with one another, as one or more cocktails, or in any sequence, in order to determine their likely efficacy against the suspected tumor tissue and cells. Thus, in some embodiments, the methods of the present disclosure include testing one therapeutic compound, or a pair of therapeutic compounds, or three therapeutic compounds, or four therapeutic compounds, or more than four therapeutic compounds, or any number of compounds in a cocktail of compounds, or provided at different times or sequences to the tissue being evaluated.

[0084] Preferably, the culturing of the tissue and the cells during the test and evaluational periods is for a length of time sufficient to obtain the values required to make a determination of the efficaciousness of one or more potential therapeutic compounds. The culturing of the tissue and the cells occurs for a period sufficiently long enough to obtain the needed data. Thus, the culturing of the tissue and the cells occurs during the one or more test and evaluational periods, and thus during a period of time of preferably less than 10 days, more preferably of less than 8 days, even more preferably of less than 6 days, and most preferably of less than 4 days. Thus, all readings typically occur within 10 days from the time of removal of the tissue or cells from the mammal. Basic microscopic readings of distance, direction and density are taken manually or by automated systems, preferably at predetermined intervals depending on the nature of the suspected tumor, the nature and amount of the tissue and the nature and number of cells or cell types being evaluated. In accordance with the various embodiments of the present disclosure, the predetermined interval is one or more hours, for example, every 4 hours, or every 8 hours or every 16 hours or every 24 hours.

[0085] Video monitoring of the tissues and cells may also be provided in order to gather and record additional information. The video monitoring is preferably provided on a continuous basis at intervals. The intervals are selected based upon the nature of the tissue and cells, the nature of the suspected tumor or tumors, and the estimation of likely behavior of the tissue and cells on a matrix. In some preferred embodiments of the present disclosure, video monitoring is provided on a continuous basis, at 30-second intervals, or at 60-second intervals or at 90-second intervals or at 120-second intervals, or at 150-second intervals.

[0086] Methods of the present disclosure include also those of testing the efficacy of therapeutic compounds on an expedited basis, testing the efficacy of therapeutic compounds with respect to a specific individual mammal, such as a human, and testing the synergistic efficacy of two or more therapeutic compounds on an expedited basis.

[0087] The present disclosure includes also one or more kits for testing the synergistic efficacy of therapeutic compounds on an expedited basis, the kit comprising i) means for collecting at least one tissue comprising cells from a mammal, the tissue being suspected of being transformed into cancerous or pre-cancerous tissue, ii) means for culturing the tissue comprising cells for a sufficient length of time to obtain desired values, iii) means for evaluating the values to determine a set of possible therapeutic compounds which might be efficacious for treating the tumor, and iv) means for testing the therapeutic effect of the determined therapeutic compounds.

[0088] As one of skill in the oncology arts will appreciate, numerous variations of the means and methods disclosed herein fall within the scope of the present application. Moreover, the examples provided herein are provided as inclusive illustrations of the present disclosure, and not as exclusive limitations.

DESCRIPTIONS OF THE FIGURES

[0089] FIGURES 1 -8 are photographic and photomicrographic images which show, by way of example, use of the means and methods of the present invention with respect to a tissue sample removed from a patient. With respect to Figures 1 - 8, Patient Sample GS-640, comprising kidney tissue, was removed from the patient by a surgeon during surgery.

[0090] Tissue analysis and screening in accordance with the invention were requested by the surgeon, and proper consent was obtained from the patient prior to surgical removal of a sample quantity of tissue from the RHS Kidney. The patient was diagnosed with

renal cell carcinoma. A tissue sample of approximately 1 gram in quantity was removed from the patient during surgery, and was placed into a 25 ml sample jar, and immersed in sterile saline. The tumor tissue was kept at 4 degrees centigrade until processed for the screening of therapeutic compounds in accordance with the means and methods of the invention. All procedures were performed under sterile conditions in a bio-safety laminar flow hood.

[0091] FIG 1: Figure 1 is a photographic image showing the mechanical division of the sample into portions to be tested. The sample has been prepared by dissecting away any non viable tissue and blood prior to mincing into 1-2 mm pieces using sterile disposable scalpels.

[0092] FIG 2: Figure 2 is a photographic image showing tumor sample portions which have been placed into each well of a 48 well plate, and been submersed in a gel matrix mixture according to the invention. After insertion of the portions, the mixture was allowed to form into a gel at room temperature for approximately 30 minutes. Once the gel formed around the fully immersed tumor fragments, nutrient rich media was added as a liquid overlay that permeated the entire gel and tumor fragment.

[0093] Each different chemotherapy and or dose of chemotherapy was added to a set of 6 wells out of the 48-well plate. A total of 7 different chemotherapies were assessed in this plate. One set of six wells were not treated with drug and thus acted as 'control conditions' for tumor fragment growth and invasion. Each of the eight vertical lanes containing six separate wells was marked with a symbol for each chemotherapy/dose used and noted correspondingly on data collection sheets. Figure 2 shows plate after chemotherapeutics and growth media have been added. The plates were then placed in a conventional 37 degree Celsius incubator at 5% CO₂.

[0094] FIG 3: Figure 3 is a photomicrographic image which shows the tumor sample at Day 0 where the initial appearance of the tissue fragment in the lower right of the photomicrograph shows no cell migration or invasion of cells away from the margins of the sample portion and out into the gel. This image is thus representative of all wells at Day 0 with or without chemotherapeutics. Images were made with a 400 X magnification phase contrast micrograph using ZEISS camera specs.

[0095] FIG 4: Figure 4 is a photomicrographic image which showing a patient control sample (no treatment) after 3 days incubation @ 37°C and 5% CO₂. Noteworthy is the migration of cells away from the solid tumor mass in a halo around the tumour fragment.

- [0096] FIG 5: Figure 5 is a photomicrographic image which shows a patient control sample after 5 days. Noteworthy is the halo of cells and increased cell density as compared with the results shown at Day 3 of the test. The lines on the images have been drawn to show the distance measurements within which approximately 95% of the migrating cells have migrated away from the margins of the tissue sample.
- [0097] Figure 5 also shows the presence of fibroblasts which sometimes appear at the later time points. Fibroblasts are noticeably different in numbers and appearance than migrating tumor cells. For example, fibroblasts appear in the surrounding gel matrix as much larger cells than the migrating tumor cells, and with significantly larger cytoplasm, as well as the classic elongated spindle fibroblast appearance. Fibroblasts also stain positively upon immuno-histochemical analysis for antibodies to fibroblast markers such as fibroblast growth factor. Thus, fibroblasts may be differentiated from tumor cells in a straightforward manner.
- [0098] FIG 6 Figure 6 is a photomicrographic image demonstrating the effect of treatment with TaxolTM. The image of Figure 6 shows a sample well at 5 days after treatment with TaxolTM. Noteworthy is the change in behavior of the cells exposed to TaxolTM. Specifically, a drop in both the distance and the number of cells that have grown and invaded away from the solid tumor fragment is seen on the lower left hand side of the image.
- [0099] FIG 7: Figure 7 is a photomicrographic image which demonstrates the effect of treatment with DocetaxolTM. The image of Figure 7 shows a sample well wherein the tumor tissue has been treated for 5 days with DocetaxolTM while showing a moderate response to the drug. This moderate response is evidenced by the fewer number of invading cells than the control but those treated with DocetaxolTM have a longer migratory distance than treatment with TaxolTM.
- [00100] FIG 8: Figure 8 is a photomicrographic image which shows the carcinoma patient sample treated for 5 Days with CisplatinTM. This image thus shows an example of a failed chemotherapy, being identical in appearance and cell numbers to the control sample (figure 5) at the same 5 day time point.

BRIEF DESCRIPTION OF THE TABLE

- [00101] The present invention is adaptable to be used with many possible therapeutic substances, and combinations thereof, including those shown in the accompanying

Table Of Exemplary Therapeutic Compounds. In accordance with important aspects of the invention, the attached Table provides only an exemplary list of therapeutic compounds which may be evaluated with the present methods and means. Virtually any other compound or combination of compounds with potential therapeutic value may be tested and evaluated with the present invention. Moreover, the means and methods of the present invention are adaptable and useful for testing any compound or combination of compounds with respect to their potential therapeutic value, regardless of whether those compounds have been involved in the drug approval process. Thus, the present invention provides means and methods for initially testing and evaluating compounds with respect to possible efficacy against many tumors and tumor types without the necessity of using animal or human subjects.

[00102] As those of skill in the art will appreciate, numerous permutations of the matrices, methods, apparatus and kits of the invention are possible within the metes and bounds of the information disclosed herein. Thus, although the present invention has been described with reference to some of the preferred embodiments, variations and modifications of steps, elements and components of the invention can be substituted therefore, while remaining within the spirit and scope of the invention.

TABLE OF EXEMPLARY THERAPEUTIC COMPOUNDS

Quantity	Compound	
3	TMZ 250mg capsules (Scering)	
1	Irinotecan 20mg/ml solution x 5ml vial (Pharmacia)	
1	Procarbazine 50mg capsule (Sigma-Tau)	
1	Methotrexate 25mg/ml solution x 20ml vial (Mayne)	
1	Carboplatin 10mg/ml solution x 45ml vial (Mayne)	
1	Adriamycin 2mg/ml solution x 100ml vial (Novo)	
1	Cisplatin 1mg/ml solution x 100ml vial (Mayne)	
1	Vincristine 1mg/ml solution x 5ml vial (Mayne)	
1	Paclitaxel 6mg/ml solution x 50ml vial (BMS)	
3	CCNU 100mg capsules (BMS)	
1	BCNU powder for injection x 100mg vial (BMS)	
1	Cyclophosphamide powder for injection x 2gm vial (BMS)	
1	Docetaxel 40mg/ml solution with diluent x 2ml vial (Aventis)	
1	5FU 50mg/ml solution x 100ml vial (Mayne)	
1	Cytarabine 100mg/ml solution x 10ml vial (Mayne)	
1	Cytarabine 100mg powder for injection (Pfizer)	

What is claimed is:

1) A method for evaluating the efficacy of one or more therapeutic compounds with respect to at least one tissue from an animal, said tissue comprising one or more types of cells, and said tissue being suspected of being abnormal, cancerous or pre-cancerous, said method comprising the steps of

A) implanting a sample quantity of said suspected tissue at least partially within a three-dimensional physiological matrix, wherein said tissue is essentially completely submerged within said matrix, said matrix being adapted and arranged for measuring one or more parameters of the behavior of said tissue and said cells,

B) providing sufficient nutrition to said cells and said tissue so that said parameters can be measured,

C) providing at least one of said one or more therapeutic compounds to said cells and said tissue,

D) incubating or culturing said tissue and said cells with said one or more therapeutic compounds in an environment suitable for the growth of said cells and said tissue for a time sufficient to obtain measurements with respect to said one or more parameters, and

E) measuring one or more of said parameters to obtain data regarding said behaviors with respect to said one or more therapeutic compounds.

2. The method of claim 1, further comprising the step of

F) from said data, determining the efficacy of said one or more therapeutic compounds with respect to said tissue and said cells.

3. The method of claim 1, further comprising the step of

G), recording said data in some form.

4. The method of claim 1, further comprising the step of

H) transmitting said data to at least one evaluator, wherein said at least one evaluator is selected from the group consisting of technicians, physicians, physician's staff members, physician's assistants, hospital employees, clinic employees, the patient, technologists, technical assistants, laboratory assistants, oncological analysts, nurses, nurses' assistants

nurse practitioners, computers, computer-aided devices, computer-facilitated devices, and agents for any of the foregoing.

5. The method of claim 4, wherein said evaluator is computerized.
6. The method of claim 1, wherein said Step C is performed during said Step D.
7. The method of claim 1, wherein said sample is divided into portions, and each of said portions is subjected concurrently and separately to said method.
8. The method of claim 1, wherein said sample quantity is divided into portions, and each of said portions is subjected concurrently and separately to said method, wherein said method includes the use of one or more controls adapted for evaluating said one or more therapeutic compounds.
9. The method of claim 7 or claim 8, wherein said measuring of Step E is performed more than once with respect to each of said separate portions of said sample, and wherein said measuring is effected during said culturing or incubating of Step D.
10. The method of claim 1, wherein said tissue is implanted in said matrix as said matrix is being formed.
11. The method of claim 1, wherein said implanted tissue is obtained from one or more of a liquid tissue, a cell suspension and cells or groups of cells which have been pelletized.
12. The method of claim 1, wherein said tissue or cells are implanted in said matrix after said matrix has formed into a gel or other permeable solid.
13. The method of claim 1, wherein said animal is one or more selected from the groups consisting of primates, humans, equines, bovines, canids, mammals, felines, canines, murines, porcines, avians, amphibians and reptilians.

14. The method of claim 1, wherein said matrix is essentially free of bicarbonate when said matrix is being formed into a gel.

15. The method of claim 1, wherein said one or more therapeutic compounds is a single compound, and said method is performed with at least one set of controls.

16. The method of claim 1, wherein said one or more therapeutic compounds are provided to said cells and said tissue as a component of said sufficient nutrition.

17. The method of claim 1, wherein said one or more therapeutic compounds are provided to said cells and said tissue as a component of said physiological matrix.

18. The method of claim 1, wherein said one or more therapeutic compounds is provided to said cells and said tissue after said sufficient nutrition is provided to said cells and tissue.

19. The method of claim 1, wherein said one or more therapeutic compounds comprises two compounds, and said method is performed with at least one set of controls.

20. The method of claim 1, wherein said one or more therapeutic compounds is three compounds said method is performed with at least one set of controls.

21. The method of claim 1, wherein said physiological matrix is adapted and arranged for measuring the efficacy of one or more therapeutic compounds, and wherein said one or more therapeutic compounds is selected from the group consisting of DNA damaging agents such as alkylating agents, antibiotics which affect nucleic acids, platinum compounds, anti-mitotics, cell cycle stimulators, anti-metastatics, anti-metabolites, camptothecin derivatives, hormone therapies, biological response modifiers, interferon, anti-invasives, radiosensitizers, radiation, and any established, theoretical or experimental therapeutics directed against cell growth or cell invasion.

22. The method of claim 1, wherein said physiological matrix is adapted and arranged for measuring the efficacy of one or more therapeutic compounds, and wherein said one or more therapeutic compounds are selected from the group consisting of Temozolomide (TMZ),

Irinotecan, Procarbazine, Methotrexate, Carboplatin, Adriamycin, Cisplatin, Vincristine, Paclitaxel, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), carmustine (BCNU), Cyclophosphamide, Docetaxel, fluorouracil (5FU), Cytarabine, doxorubicin, bleomycin, topotecan, tamoxifen (TMX), and imatinib mesylate (Gleevec) and all other therapeutics appropriate for treatment.

23. The method of claim 1, wherein said one or more therapeutic compounds comprises at least two therapeutic compounds.

24. The method of claim 23, wherein said at least two therapeutic compounds are selected from one or more of CAF (Cyclophosphamide/ Adriamycin/Fluorouracil); CMF (Cyclophosphamide/ Methotrexate Fluorouracil); CMFVP (Cyclophosphamide/ Methotrexate/Fluorouracil/ Vincristine / Prednisone); PCV (Procarbazine/CCNU/ Vincristine); ICARBO-E (Ifbsfamide/Carboplatin/Etoposide); TAP (Taxol/Adriamycin/ Cisplatin); EMA-CO (Etoposide/Methotrexate/Actinomycin/Cyclophosphamide/ Vincristine); VBP (Vinblastine/Bleomycin/Platinol (Cisplatin)); BPD-T: (Bcnu/Platinol/ Dacacarbazine/ Tamoxifen); and T-IO (Methotrexate/Bleomycin/Cyclophosphamide/ Dactinomycin/Adriamycin) and all other therapeutics appropriate for treatment.

25. The method of claim 1, wherein said method is adapted and arranged to test the efficacy of non-synthetic chemical therapies.

26. The method of claim 1, wherein said non-synthetic chemical therapies are one or more selected from the group consisting of radiation therapies, brachiotherapies, herbal therapies, naturopathic therapies, experimental therapeutic compounds, and new therapeutic compounds.

27. The method of claim 1, wherein said matrix is essentially free of bicarbonate when said matrix is being formed into a gel or a permeable solid.

28. The method of claim 1, wherein said matrix is formed from components, wherein said components are essentially free of bicarbonate.

29. The method of claim 1, wherein said measuring of Step D is performed at periodic intervals, and wherein said periodic intervals are appropriate to one or more of the cells, the tissue and the therapy or therapies being tested or evaluated.

30. The method of claim 1, wherein said measuring of Step D is performed at periodic intervals for a period of time of between one hour and 15 days.

31. The method of claim 30, wherein said periodic intervals are one or more selected from the group consisting of every six hours, every 12 hours, every 18 hours, every 24 hours, every 36 hours, every 48 hours, every 72 hours and every 96 hours.

32. The method of claim 30, wherein said periodic intervals are one or more selected from the group consisting of every day, every second day and every third day, every fourth day, every fifth day, every sixth day, every seventh day, every eighth day, and every ninth day.

33. The method of claim 1, wherein said measuring of Step D is performed at non-periodic intervals, and wherein said non-periodic intervals are appropriate to one or more of the cells, the tissue and the therapy or therapies being tested or evaluated.

34. The method of claim 1, wherein said data obtained by the measuring in Step D is recorded in a fixed media.

35. The method of claim 34, wherein said fixed media is one or more selected from the group consisting of paper, voice recordings, video recordings, photographs, photomicrographs, digital recordings, any fixed digital data means, any computer network-facilitated means, infrared recording, ultrasound recordings, and magnetic resonance recordings.

36. The method of claim 1, wherein said data is obtained by one or more of a human observer, a still camera, a video camera, an automated still camera, an automated video camera, an infrared camera, and an automated infrared camera.

37. The method of claim 1, wherein said culturing of said tissue and said cells occurs for a period of time sufficient to obtain said data.

38. The method of claim 1, wherein said culturing or incubating of said tissue and said cells occurs for a period of time of between one hour and 15 days.

39. The method of claim 1, wherein said culturing or incubating of said tissue and said cells occurs for a period of time of at least 1 hour, or at least 10 hours, or at least 24 hours, or at least 36 hours or at least 72 hours, or at least 125 hours, or at least 175 hours, or at least 200 hours, or at least 250 hours or at least 300 hours.

40. The method of claim 1, wherein said sufficient nutrition comprises one or more fluids, and wherein said one or more fluids are selected from the group consisting of tissue culture medias, concentrated tissue culture medias, and any culture or nutritional medias which are suitable for said tissue sample.

41. The method of claim 40, wherein said concentrated medias are one or more selected from the group consisting of 1OX medias, and any other concentrated medias.

42. The method of claim 1, wherein said three-dimensional physiological matrix comprises said sufficient nutrition.

43. The method of claim 1, further comprising the step of

l) providing at least one source of additional nutrition to said tissue and said cells so that said parameters can be measured.

44. The method of claim 43, wherein said at least one source of additional nutrition comprises one or more of tissue culture medias, concentrated tissue culture medias, and any culture or nutritional medias which are suitable for said tissue sample.

45. The method of claim 44, wherein said at least one additional source of nutrition is supplemented with one or more selected from the group consisting of comprising sera, proteins, sugars, salts, lipids.

46. The method of claim 1, wherein said sufficient nutrition is provided to said tissues and said cells by placing said sufficient nutrition in contact with said matrix after said sample

has been placed in said matrix, and said matrix has equilibrated to room temperature, wherein said room temperature is in the range of from 10 to 30 degrees Celsius.

47. The method of claim 1, wherein said matrix is formed to comprise said sufficient nutrition.

48. The method of claim 1, wherein said environment for incubating or culturing said cells and said tissue includes a temperature range of from 26 to 43 degrees Celsius, a carbon dioxide tension of from 2.0% to 9.0%, and a humidity range of from 50-100 %.

49. The method of claim 1, wherein said sample quantity is divided into portions, and each of said portions is subjected concurrently and separately to said method.

50. The method of claim 1, wherein said matrix comprises a gel, and wherein said gel is suitable for measuring said one or more parameters to obtain said values.

51. The method of claim 1, wherein said matrix is formed in multiple containers such that multiple portions of said sample can be evaluated.

52. The method of claim 1, wherein said matrix is formed in multiple containers and said multiple containers comprise a multi-well apparatus or plate.

53. The method of claim 1, wherein said one or more parameters comprises one or more of: the distance which individual cells migrate from said tissue sample, the average distance of migration of a group or population of said cells from said tissue sample, the distance which individual cells migrate from said tissue sample with respect to time, the average distance of migration of a group or population of said cells from said tissue sample with respect to time, the velocity of migration of one or more designated individual said cells per selected time period, the velocity of migration of a group or population of designated said cells per selected time period, the number of migrational cells per unit area of a microscopic visual field, the speed of proliferation of said cells, the speed of unidirectional migration of said cells, the speed of bi-directional migration of said cells, the speed of tri-directional migration of said cells, the frequency of directional change of said migrating cells, the number of directional changes per unit time of said migrating cells, the rate of mitosis of said migrating cells, the

number of cells migrating per unit time, the number of cells in a unit area of a portion of the gel, the change in the number of cells in a unit area of a portion of the gel with respect to time, the number of cells in a unit volume of a portion of the gel with respect to time, and the change in the number of cells in a unit volume of a portion of the gel, the proportion of cells which change migrational direction during the test period, the speed of migrating cells in a particular visual field, the density of migrating cells per unit area of a visual field, the migrational distance of individual migrating cells per unit area of a visual field, the average migrational distance of a group or population of migrating cells per unit area of a visual field, and the number of migrating cells in a particular direction per unit area of a visual field.

54. The method of claim 1, wherein said physiological matrix has a pH in the range of from 5.0 to 8.0.

55. The method of claim 1, wherein said physiological matrix has a pH in the range of from 7.2 to 7.6.

56. The method of claim 1, wherein said matrix comprises at least one natural or synthetic fiber.

57. The method of claim 1, wherein said at least one natural or synthetic fiber is one or more selected from the group consisting of type I collagens, type II collagens, type III collagens, type IV collagens, fibrin, fibrinogen, extracellular matrix proteins derived from one or more animals, laminin, fibronectin, anti-laminin, and any other natural or synthetic fibers or cables suitable to the formation of a matrix mesh or network capable of supporting cell support, movement and growth.

58. A method for evaluating the efficacy of one or more therapies with respect to at least one tissue from an animal, said tissue comprising one or more types of cells, and said tissue being suspected of being abnormal, pre-cancerous or cancerous, said method comprising the steps of

A) implanting a sample of tissue from said animal at least partially within a three-dimensional physiological matrix, wherein said tissue is essentially completely submerged within said matrix, said matrix being adapted and arranged for measuring one or more parameters of the behavior of said cells of said tissue or cells,

B) providing sufficient nutrition to said cells of said tissue of said portions so that said parameters can be measured,

C) subjecting said cells and said tissue to at least one of said one or more therapies

D) incubating or culturing said tissue and said cells of said portions in an environment suitable for the growth of said cells and said tissue for a time sufficient to obtain measurements with respect to said one or more parameters for each of said portions, and

E) measuring, with respect to each of said portions, one or more of said parameters to obtain data regarding said behaviors with respect to said one or more therapies.