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(71) Applicant *(for all designated States except US)*: **FRAUNHOFER-GESELLSCHAFT ZUR FÖRDERUNG DER ANGEWANDTEN FORSCHUNG E.V.** [DE/DE]; Hansastrasse 27c, 80686 München (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WALLES, Heike** [DE/DE]; Bahnhofstrasse 21, 71063 Sindelfingen (DE). **HEYMER, Andrea** [DE/DE]; Ulmenstrasse 5, 71720 Oberstenfeld (DE). **HANSMANN, Jan** [DE/DE]; Neubauerweg 10, 70569 Stuttgart (DE). **KAUFMANN, Michaela** [DE/DE]; Oelkinghauser Strasse 14, 58332 Schwelm (DE). **REIS, Christian** [DE/DE]; Blaulstrasse 11, 67346 Speyer (DE). **HUCHLER, Roland** [DE/DE]; Klugestrasse 50, 70197 Stuttgart (DE). **TRAUBE, Andreas** [DE/DE]; Florianstrasse 1, 72622 Nuertingen (DE). **STALLKAMP, Jan** [DE/DE]; Paracelsusstrasse 35/2, 70771 Leinfelden-Echterdingen (DE). **PRETZSCH, Frank** [DE/DE]; Waldorferstrasse 8, 50969 Koeln (DE). **DERICHS, Christian** [DE/DE]; Prinzenhofstrasse 23, 52062 Aachen (DE). **KOROPP, Ulrike** [DE/DE]; Wilhelmstrasse 53, 52070 Aachen (DE). **MARX, Ulrich** [DE/DE]; Brahmsstrasse 5b, 53121 Bonn (DE). **PILGERMANN, Stefan** [DE/DE]; Drieschgaerten 25, 52382 Niederzier (DE). **SAXLER, Joerg** [DE/DE]; Eilpeweg 25a, 45277 Essen (DE). **STOLZING, Alexandra** [DE/DE]; Taeubchenweg 43b, 04103 Leipzing (DE).

(74) Agents: **SCHWAHN, Hartmut** et al.; Gleiss Grosse Schrell & Partner, Leitzstrasse 45, 70469 Stuttgart (DE).

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(54) Title: MODULAR SYSTEM FOR THE AUTOMATIC PRODUCTION OF THREE-DIMENSIONAL TISSUE STRUCTURES

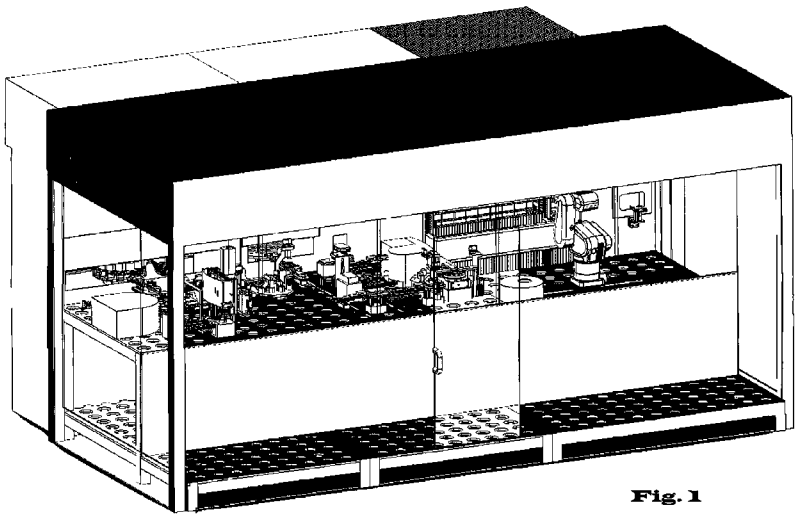

Fig. 1

(57) Abstract: The invention resides in the technical field of tissue engineering and relates to the automatic production of three-dimensional, newly constituted biological tissue structures made up of individual cells or tissue clusters. The invention provides an automated modular production system and a controlling system therefor.

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Modular system for the automatic production of three-dimensional tissue structures

Description

The invention relates to the automatic production of three-dimensional, newly constituted biological tissue structures made up of individual cells or tissue clusters. The invention provides for this purpose an automated modular production system and the controller therefor.

The invention resides in the technical field of tissue engineering. The principle of tissue engineering consists substantially in isolating vital cells or cell clusters from biological tissue which can be recovered, for example in a separate method sequence, from the human or animal body in the form of donor tissue particularly known as biopsates (biopsy specimen). The isolated cells are propagated and subsequently applied in order to build up newly constituted three-dimensional tissue structures, what are known as artificial tissues or tissue equivalents, for example newly constituted skin equivalents. The build-up or constitution of skin test systems requires two different primary cell types: fibroblasts and keratinocytes which, as is known, are isolated from multilayered skin tissue, in particular prepuce biopsates. Newly constituted tissues of this type can then be used as test systems in research, in particular for researching active ingredients or as transplants in medicine, in order to replace lost organ functions. For ex-

ample, three-dimensional, i.e. two-layered skin equivalents are used as test systems for active ingredients, chemicals and cosmetics and in this way provide an alternative to animal testing.

The high demand for artificial tissue, in particular for skin test systems produced from preferably human primary cells, cannot be met by individual manual processing and the requirements placed on the reproducibility thereof cannot be fulfilled. It is expected that this can be ensured only by automation of the production process.

In the production of three-dimensional tissue structures made up of individual cells or tissue clusters, there is a demand for high-quality tissue models built up from primary cells or cell lines. In the past it has not been possible to economically and rapidly provide validated *in vitro* test systems in a satisfactory manner.

The difficulties in automation reside in the fact that the complex sequences of different manual steps of cell isolation, cell cultivation and tissue build-up cannot simply be replaced by automatable handling operations representing the manual process. However, the particular challenge facing a production system for the automated production of tissue resides in the superordinate interfaces of the plant and in controlling the flows of substances; no satisfactory solution has to date been found for this problem.

For a use of tissue engineering products in regenerative medicine, there are also legal regulations with regard to sterility and reproducible quality that it is possible to comply with only under clean room conditions and by means of validated processes. For manufacturing transplants, cross-contaminations of different samples must be avoided by way of a complete batch separation. When dealing with living biological materials, the

following precisely predefined marginal conditions must be adhered to: Sterile conditions must prevail within the fully automated plant. As soon as transplants for use on human beings are manufactured in the plant, GMP guidelines stipulate that Class A clean room conditions must be adhered to. All the solid, liquid and gaseous, materials used may be introduced into the plant only when sterile. Maintaining sterility requires regular cleaning and sterilisation cycles. Under GMP guidelines, a maintenance access must fulfil the Class B clean room conditions. Solid waste such as disposable materials and also liquid solutions must be removed from the path of production without giving rise to a risk of contamination. No waste may be stored within the plant.

In the manufacture of transplants, it must at all times be possible to locate or monitor the cells of a donor in the production site. There should be no risk of mixing-up of the samples or of cross-contamination.

In addition, owing to the diversity of biological samples, it is not possible to specify the number of isolated vital cells to be expected in the biopsate beforehands. This requires the design of an automated method sequence in such a way that a maximum number of biopsates and cells can be processed per day in order to be sure of achieving the minimum quantity of cells required for daily production, even if only a relatively small number of vital cells can be isolated from each individual biopsate. This requirement is pitted against the limited capacity of the processing steps ensuing in the process as a whole. It is also necessary to ensure that the vitality of the cells is not impaired during the transfer from processing step to processing step. The number of intermediate steps for the intermediate storage of material should be minimised or avoided altogether.

Ideally, it should be possible for the tissue which is produced, both for use as a test system and for transplantation, to be packaged in a sterile manner and checked for sterility and quality. In order to be able to ensure a high-quality product, it should be possible to subject the tissue products to quality control after they have passed through the production chain.

Previously attempted solutions for automated systems in the field of tissue engineering have been restricted to the isolation or to the cultivation of cells. Use is made of standardised bioreactors or of specially adapted surfaces or membranes in reactors. There are no equipment-based solutions for the integrated isolation, cultivation and build-up (constitution) of vital three-dimensional artificial tissues.

The technical problem underlying the invention consists in providing methods and means for carrying out these methods that allow automated production and control and process control for operating the automated production of three-dimensional tissue structures made up of individual cells or cell clusters (tissue engineering).

In a first aspect, the invention provides a fully automated production system or plant for the production of multilayered three-dimensional tissues which are suitable for use as test systems or as transplants, which system is constructed in modules. In the context of this invention, the term „module“ refers to an arrangement of device elements and handling elements which are together suitable and preferably specifically embodied for carrying out at least one self-contained process step in the production according to the invention of three-dimensional tissues.

According to the invention, the modules of the plant are arranged sequentially next to one another in accordance with the processing sequence comprising 1. cell extraction, 2. cell expansion and 3. tissue build-up (con-

stitution), preferably not spatially separated from one another, preferably directly adjoining one another.

The at least three core modules of the modular device or plant are:

- a cell extraction module in which the primary cells can be isolated from the donor tissue (first step);

- a cell expansion module in which the isolated cells can undergo maximum propagation in a controlled manner (second step); and

- a tissue constitution module in which the cells can be built up to form a three-dimensional multilayered tissue (third step).

In a preferred variant thereof, a combination with further modules is provided, in particular for extending the functional scope of the plant. In an alternative variant of the invention, the modular construction allows one or more of the individual modules to be operated in a stand-alone manner.

In one embodiment, the modules are each connected to one another via interfaces allowing communication and flow of material between the modules. Preferably, provision is made for each of the at least three modules to have standardised outward interfaces, particularly a separate material lock.

In one embodiment each module has at least one module control computer which is integrated into the module or associated with the module. It preferably controls and partly regulates the sequences in the respective module, including the control of flows of substances, management of supplies of material and media, determination of status variables of the module and, if appropriate, generation of error messages.

As the processes within the modules are dependent on the processes in the other respective modules, the flows of material between the modules and/or within the modules are regulated. According to the invention, the module control computers which are preferably provided are data-connected to a central control computer remote from the module. The central control computer regulates functional interlinking and the flow of material between the modules, preferably by controlling module-individual timing and/or turnover of material, as a function of at least one state variable of the modules that is communicated from the module control computers to the central computer.

The invention also provides methods and means for the control and process control of these modules.

The subject matter of the invention is accordingly a method for the automatic production of biological tissue newly constituted from animal or human donor tissue, including the steps:

- providing the donor tissue at an input interface of a first module which is specifically embodied to isolate from the donor tissue donor tissue cells of at least one cell type and to single out these cells (cell extraction module), so that singled-out cells of the at least one cell type that are isolated from the donor tissue are obtained;

- transferring the isolated cells at a first interface of transition (transition point) to a second module which is specifically embodied to propagate the respectively isolated cells of the at least one cell type (cell expansion module), so that in each case at least one cell group made up of propagated cells of the at least one cell type is obtained from the isolated cells;

- transferring the at least one cell cluster at a second interface of transition (transition point) to a third module which is specifically embodied to newly constitute a biological tissue with one or more of the cell groups (tissue constitution module), so that a newly constituted biological tissue is obtained from one or more cell groups of at least one respective cell type; and

- providing the newly constituted biological tissue at an output interface.

In the context of this invention, the term "cell group" refers in an embodiment to substantially or completely contiguous cells which are cultivated, in particular, to confluence or almost to confluence and are made up of propagated, in particular previously originally singled-out seeded cells of a cell type. In an alternative embodiment the term refers to a suspension made up of, if appropriate, resuspended cells of a cell type that are propagated from individual cells by cultivating.

Preferably, the process control within the modules is carried out by module control computers respectively associated with the modules.

Preferably, however, the first module has between the input interface and first transition interface a first material flow rate; the second module has between the first transition interface and second transition interface a second material flow rate and the third module has between the second transition interface and output interface a third material flow rate. According to the invention, preferably, one or more of the first, second and third material flow rates is controlled as a function of at least one material flow rate different therefrom, selected from the first, second and third material flow rate, and/or of at least one state variable of at least one of the first, sec-

ond and third modules in order to adapt the material flow rates of the modules.

Preferably, the material flow rate is controlled by controlling the timing of the module.

In the context of the invention, the term "timing" primarily refers to the process speed of the process steps and handling operations occurring within the module. The process speed within the module can be controlled in particular by way of the speed of individual method steps carried out within the modules and/or based on the interval times of material handling between the actual process steps. In this respect, the interval times between the method steps may have the effect of an "intermediate storage" of the processed material within the process sequence. This "storage" is different from what is commonly known as „intermediate buffering", but may be as effective. The latter being a further variant and comprises at least one particular separate method step within the process sequence that serves to intermediately store or to collect a material flow for a specific duration before the material is subjected to further process steps. In a variant intermediate buffering preferably takes place in the „main stream" of the material flow, wherein substantially all of the material in the process is brought into the intermediate buffer and no subsequent process step is carried out until the intermediate buffering method step has been completed. In an alternative variant the intermediate buffering follows in the "side stream", wherein only a predetermined fraction of material is intermediately buffered, the other (main) fraction being further processed immediately in the subsequent process steps. In a preferred embodiment the overall material flow rate in that module within a module is therefore adapted by intermediate buffering of the material flow. In a preferred variant the extent of the intermediate buffering and thus of the material flow

within the module is controlled by controlling the duration of the intermediate buffering, in particular in conjunction with the intermediate buffering in the „main stream" of the material flow. In an alternative preferred variant the extent of the intermediate buffering is controlled by controlling the ratio of the amount of material intermediately buffered in the side stream and the amount of material which is immediately further processed in the main stream.

The invention includes plurality of variants for controlling and regulating the material flow within the modules. In a first variant the material flow rate in the first and/or second module is controlled as a function of the cell type processed in the third module.

In a further variant the material flow rate in the first module is controlled as a function of the number of cells propagated in the second module.

In further, alternative or additional, variants the material flow is controlled by one or more state variables. In one variant the state variable is the cell count.

In a further variant the state variable is the cell density.

In a further variant the state variable is the cell vitality.

In a further variant the state variable is the rate of proliferation of the cells.

Preferably, the cell density is determined by measuring the transepithelial electrical resistance (TEER). Preferably, the cell vitality is automatically detected via optical measurements, in particular by applying a vital dye under microscope control using image recognition software. Alternatively preferred are spectroscopic measurements, particularly preferably via

Raman spectroscopic analyses, preferably compared to reference spectra. The rate of proliferation of the cells or cell count of the propagated cells detached from the reactor is preferably determined by means of a cell counting chamber, wherein the total cell count is back-calculated from the sample brought into the counting chamber.

The invention provides the merging of self-contained stand-alone process sequences which each occur separately per se to a combined system.

The automatic isolation of cells from the donor tissue in a first module is thereby carried out preferably in at least the following partial steps:

- automatic separation of fatty tissue which may be present on the donor tissue from the cells to be isolated;

- automatic incision for breaking open the tissue structure of the donor tissue containing the cells to be isolated;

- automatic incubation of the donor tissue with the broken-open tissue structure in enzyme solution for separating a cell cluster of a first cell type;

- automatic separation of the individual cells detached from the cell cluster of the first cell type of the incubated donor tissue from the residual tissue of a further cell type; and

- automatic resuspension of the individual cells; so that a suspension of cells of a cell type that are isolated from the donor tissue is obtained.

In a preferred variant the method is supplemented at least by the following further partial steps:

- automatic incubation of the residual tissue containing cells of the further cell type in enzyme solution for breaking up the cell group of the residual tissue into individual cells of the further cell type,

- automatic resuspending of the cells of the further cell type detached from the cell cluster of the residual tissue.

Automatic propagation of isolated cells in the second module to form a cell group is carried out in at least the following partial steps:

- determining the cell count of the isolated cells based on a cell sample taken from the suspension of the isolated cells in a counting device, in particular a counting chamber,

- setting the cell count of the isolated cells in the suspension to a predeterminded cell concentration,

- seeding the isolated cells in the predeterminded concentration, if appropriate aliquoting the cells in a predetermined number, into a bioreactor,

- incubating the cells bioreactor with the seeded thereon or therein for cell propagation.

In a preferred variant, this method is supplemented at least by the following further partial steps:

- singly or multiply determining cell cultivation parameters, selected from pH, oxygen partial pressure, TEER, glucose content, opacity, cell count, and cell density, before, during and/or following the incubation.

Preferably, a cell count is determined before the incubation. It is preferable for a preferably recurring determination of the parameters, selected from TEER, glucose, oxygen partial pressure, pH and opacity, to be carried out during the incubation. Preferably, cell counting is carried out, preferably repeated, after the incubation, i.e. at the end of the culturing period.

The automatic build-up of tissue (constitution) from at least one cell group of propagated cells in the third module is preferably carried out in at least the following partial steps:

- automatic bringing of a tissue carrier into contact with a first layer of the cell group,

- incubating the layered construct thus produced in order to constitute a newly constituted biological tissue.

In a preferred variant this method is supplemented at least by the following further partial steps:

- applying a second or further layer of a cell group of propagated cells of a further cell type to the first layer of cells.

In a preferred further partial step the tissue quality of the tissue construct thus produced is determined, preferably by means of optical coherence tomography (OCT).

In a preferred embodiment, the donor tissue or biopsate is examined for biological and/or chemical contamination before entering the process modules, especially through the input interface of the first module. Methods known per se may be used for this purpose. In a first preferred variant a cultivating culture may be started and incubated for this purpose. The

growth of microorganisms is detected in a manner known per se and the microorganisms found are specified if appropriate. In an alternative or preferably additional carried-out variant the biopsate itself and/or the supernatant of the culture medium above the biopsate is subjected to a spectroscopic, in particular Raman spectroscopic spectral analysis, and contamination can be concluded and if appropriate the type of contamination can be specified, preferably by comparing the spectra found with reference spectra. The vitality and/or the degree of differentiation of the cells contained in the donor tissue can if appropriate also be concluded from the spectra found.

The invention makes provision for the donor tissue preferably to be a biopsate of the human or animal body, preferably multilayered skin tissue. The type of cell to be isolated is selected from fibroblasts and keratinocytes.

However, the invention is not limited to these cell types. The person skilled in the art can transfer the principles of this invention also to application in other tissues and other cell types without inventive activity; he may be well aware of the adaptations necessary for his purpose, as soon as he has understood the invention on the basis of the cell types, fibroblasts and keratinocytes, that are mentioned herein in connection with the use of a skin biopsate by way of example.

Such newly constituted biological tissue according to the invention may be a multilayered tissue which contains preferably at least one cell layer made of fibroblasts and preferably at least one cell layer made of keratinocytes.

The subject matter of the invention is therefore also an artificial multilayered skin tissue construct which is producible or is preferably produced using the method according to the invention.

The subject matter of the invention is also a device for the automatic production of biological tissue which is newly constituted from animal or human tissue and comprises at least the components mentioned hereinafter:

- a first module (cell extraction module) which is specifically embodied to isolate from the tissue a tissue cell of at least one cell type and to single out these cells, which has an input interface and a first transfer interface for transferring the isolated cells,

- a second module (cell expansion module) which is specifically embodied to propagate the isolated cells of the in each case at least one cell type, the second module being connected downstream of the first module, sharing therewith the first transfer interface and having a second transfer interface for transferring the group of propagated cells; and

- a third module (tissue constitution module) which is specifically embodied to newly constitute a biological tissue with the at least one cell group, the third module being connected downstream of the second module, sharing therewith the second transfer interface and having an output interface for ejecting the newly constituted biological tissue.

The device may comprise at least one control unit for detecting material flow and/or state variables of at least one of the modules, which module is specifically embodied to control the material flow in at least one module as a function of the material flow and/or at least one state variable of at least one further module.

In one preferred embodiment the first module comprises at least the following elements for carrying out the cell extraction:

- a fat separator device for the separating of fatty tissue which may be present on the donor tissue from the donor tissue,

- a chopper device for cutting into the tissue structure of the donor tissue,

- an incubator for incubating the donor tissue with the cut-into tissue structure, and

- a pipetting device for the separation of cells to be isolated from the donor tissue from residual tissue.

In one preferred embodiment the second module comprises at least the following elements for carrying out the cell expansion:

- a pipetting device for holding the isolated cells in suspension and for metering the cell suspension into or onto a bioreactor,

- a cell counting device for determining the cell count in the cell suspension, and

- an incubator for incubating the isolated cells seeded on or in a reactor.

In a preferred variant, the second module also comprises:

- a measuring station for determining the state parameters of the seeded cells cultivated in or on the bioreactor and/or

- a media exchange station for exchanging the cell culture medium and/or for incubating the cells.

In one preferred embodiment the third module for carrying out the tissue build-up (constitution) comprises at least the following elements:

- a handling device for applying cultivated cell clusters to a tissue carrier,

- a measuring device for determining the tissue quality, and

- an incubator for incubating the tissue carrier and the cell cluster applied thereto.

The entire modular plant may be used in such a way as to build up from donor tissue fully automatically, and in particular in a robot-assisted manner, three-dimensional multilayered biological tissue constructs, particularly test systems made up of various cell types. In consideration of the legal requirements, the plant may be operated under GMP conditions and may thus be preferably used for the manufacture of autologous and/or allogenic transplants for medical use. According to this invention, a complete automation is provided that offers surprisingly increased product safety and reproducibility when compared to common manual production processes in GMP-compliant laboratories. The modular construction also allows individual modules, such as the cell extraction module, the cell expansion module, or elements of the modules, such as the multifunctional pipette, the tissue grinder, to be operated individually in a flexible and stand-alone manner.

Before being introduced into the plant, the biological donor material may be thoroughly rinsed in a separate GMP laboratory or under a separate laminar flow and a sample of the rinsing buffer is taken. This sample is then incubated for at least 48 h in an incubator at 37 °C and on the following day checked visually for contamination.

After passing through the production chain, all the tissue products are subjected to a quality control. It is possible to be able to carry out non-invasive, in particular contactless, non-destructive, measurements of the inner structure of the tissue products by means of optical coherence tomography (OCT). It is thus not necessary to produce any destructive histologies therefrom. Defects and inhomogeneities in the tissue structure may be detected and evaluated in a fully automatic manner using the OCT technique. This allows a 100 % in-line quality control fo the product at the end of the production chain.

In one preferred embodiment the invention makes provision for one or more of the modules each to have at least one module control computer for respectively controlling the processes in the module. Furthermore, provision is made for the at least one module control computer to serve to determine the material flow rate and/or at least one state variable of the module.

In one preferred embodiment the at least one module control computer is in data-connection with a central control computer. That means particularly that the module control computer is suitable for, and in a preferred variant specifically embodied for, transmitting at least one module-internal variable, inter alia process parameter, material flow rate, state variable, detected in the associated module to the central control computer.

Furthermore, particularly the central control computer is suitable and preferably specifically embodied for controlling via at least one module control computer the material flow rate and if appropriate other process conditions in the module associated with the module control computer, and in particular to regulate these as a function of one or more module-internal variables.

The subject matter of the invention is also a computer program product for controlling the material flow in the modular device of the invention characterized herein, wherein the first module comprises between the input interface and first transition interface a first material flow rate, the second module comprises between the first transition interface and second transition interface a second material flow rate and the third module comprises between the second transition interface and output interface a third material flow rate. The computer program is characterized by the program steps:

- detecting one or more of the first, second and third material flow rates and/or one or more state variables of the first, second and third modules;

- controlling at least one first, second and third material flow rate different therefrom, as a function of the detected at least one material flow rate and/or state variable in order to adapt the material flow rates of the modules to one another.

In the following, the invention is describes in more detail without being limited to the following embodiements: The modules are preferably arranged under a laminar flow box or an arrangement similar thereto in order to fulfil the clean room Class A requirements. The modules are outwardly demarcated preferably by locks arranged on a side (front) of the modules. In addition, maintenance accesses (clean room Class B) are preferably provided at the back. The equipment and means for handling all the modules are located preferably at the level of a preferably perforated carrier bench (perforated table) which is preferably made from special steel. Below the table, space is preferably provided for the periphery of the plant or for control and waste disposal.

In order to ensure the sterility of the processes, at least those parts of the plant in which open cells and tissues are worked on are preferably placed under a clean room Class A laminar flow, preferably having a low-turbulence displacement flow rate of approximately 0.45 m/s. Preferably, a clean room Class B laminar flow and a pressure which is preferably about 12 Pa higher than in the ambient environment prevail in the outer regions containing the material locks and maintenance accesses. This difference in pressure reduces the risk of contamination in the plant. In order to reduce the risk of contamination, daily, automatically occurring separate sterilisation cycles, during which no tissues or cells are processed, are preferably scheduled.

Within this period the modules are preferably sterilised by means of what is known as Minncare Dry Fog® technology MINNTECH, Cantel Medical Co. USA). This technology is an aerosol-based clean room disinfection using dry fog, in which all the surfaces entering into contact with the air are disinfected without the need for recleaning or the surfaces of the module components becoming damaged.

The modules preferably have in each case at least one separate material access. Preferred conditions are in this case for all the materials, both solid materials and liquids or gases, to be introduced in a sterile manner into the plant via the material locks. The material locks preferably have a sterilisation unit. An engineer can therefore at the beginning of production load the material locks with the required material, subsequently close them and sterilise the material for this purpose. The handling units from the inner regions of the modules can subsequently integrate the materials, which are thus completely externally and internally sterile, into the process. Liquid or gaseous media are preferably supplied also via the material locks. Preferably, supply bottles, bags or canisters with a septum or click

closure are introduced, which are then connected in an automated manner under sterile conditions by a respective dispensing unit which is preferably associated with the module and particularly preferably contained in the module and the liquids can then be forwarded into the plant via hoses.

In addition, the modules preferably have in each case at least one separate maintenance access. In an emergency or within predefined time windows it is also possible to intervene in the inner part of the modules through the accesses which are embodied as locks. For more extensive maintenance or repair work, further maintenance accesses, via which waste can also be disposed of, are located on the plant. It is possible to enter the maintenance accesses from the outside via a door. In particular during a production of transplants for use on human beings under GMP conditions, a lock for persons, through which staff can pass from a surrounding Class C clean room region into the clean room Class B maintenance region, is preferably constructed in front of the maintenance access.

For the disposal of waste from the modules, the invention preferably makes provision for solid waste, such as disposable materials, and also liquid solutions to be collected in specific containers below the equipment table toward the maintenance access and to be disposed of in regular cycles, e.g. once per day, via the maintenance access.

The bioreactor systems designed for use in the modules are preferably made in the standardised format of the microtitre plates which are known per se in order to be able to draw on standard handling solutions. Preferably, the individual marking of the reactor plates is provided with barcodes or RFID or a similar system. This is intended to ensure monitoring and in particular to prevent cross-contaminations.

Preferably, a special multifunctional pipette described hereinbefore in relation to the first module is used for handling cell suspensions. At the same time, the multifunctional pipette can replace the centrifugation, i.e. individual cells can be separated from liquids. Furthermore, cells can be resuspended and homogeneously distributed in a liquid. Cells are preferably also transferred between the modules with the aid of this pipette.

Provision is preferably made for the timing within the plant to take account of the marginal conditions defined by the biology and the capacity of the modules and to allow adequate delivery of the plant components with the required materials from the lock. As a result of the sequential processing, which is preferably provided in accordance with the invention, in the cell extraction module, feedback from the cell expansion module is preferably responded to and the isolating process is in this way stopped, for example in the case of a sufficient cell count in the cell expansion module. This allows resources to be saved.

On account of the predefined protocol for sequentially constructing the skin models, the transfer from the cell expansion module to the tissue constitution module is preferably carried out as follows: The tissue constitution module is preferably configured in such a way that it requires and preferably demands specific quantities of cells at precisely defined times in order to be able to reproducibly construct the tissue models.

The process management and timing in the cell expansion module is preferably oriented in such a way as to be able to supply the tissue constitution module with cells at specific times while at the same time it remains possible to carry out the module-internal handling steps (exchange of media, seeding of cells from the cell extraction module) without unnecessary intermediate buffering. For this purpose, provision is preferably made for

firstly cell type 1, in particular fibroblasts, to be transferred at regular intervals from the cell expansion module to the tissue constitution module. These time intervals are preferably selected in such a way that the handling means in the tissue constitution module can carry out all the required steps for building up the first tissue layer, in particular dermis. Preferably after this has been completed, the delivery with cell type 2, in particular keratinocytes, takes place for building up the second tissue layer, in particular epidermis. It is thus possible to advantageously ensure that in the same portion of time, for example one day, the same quantity of cells can be isolated and further processed and the overall plant can produce in this portion of time roughly the same number of finished skin models, of which each skin model was produced substantially in exactly the same way. A huge rise in quality is surprisingly achieved as a result.

An optimisation, which is preferred in accordance with the invention, in the process sequence is related to the limited durability of the donor tissue, the avoidance of drying-out of the donor tissue during processing and the predefined incubation times in enzyme solutions, on the cultivation times and the predefined order of tissue build-up. The limited durability of the biopsates is preferably improved by speeding up the preceding sterile control, e.g. by means of automated Raman spectroscopy. As a result of the complete automation according to the invention, the cell extraction process proceeds more rapidly than the manual laboratory process. This minimises the risk of the biopsate drying out during the processing process.

The sequential timing between the modules allows the intermediate storage, which may be required, of the cells in the transport medium to take place up to a defined non-critical period of time.

A further optimisation consists in the media guidance and temporal process control during the build-up of the first layer of the tissue constructs. Separate media guidance, which is provided in accordance with the invention, of components of the tissue constructs allows an undesirable early formation of gel to be prevented. One component reproduces the natural extracellular matrix, while the other component functions as a cell carrier. When both components meet, a chemical reaction ensues leading to rapid hardening of the two liquids. Premature hardening of the cell suspensions in the metering systems is in this way prevented.

A further optimisation consists in the storage of cells or tissues. If there is overproduction or an excessively low demand, cells and/or tissues can be guided from the plant and be preserved and stored for a relatively long time in accordance with various cooling protocols in an external cryomodule.

For an overview of the processes occurring within the modules, digital cameras are preferably positioned at various points. Preferably, the number of particles in the air is monitored at defined points in the plant, preferably by means of automatic particle counting apparatuses.

The invention makes provision for the timing of the individual modules to be adapted to one another. Preferably, relevant information concerning the process is displayed and/or stored in a GMP-compliant manner. The individual production processes are documented in preferably predefined formats. Critical process steps are monitored. Provision is made for the central computer to respond to error messages and to initiate appropriate measures to eliminate errors and/or compensate for errors in the individual modules. The use of suitable sensors is intended to implement automated process control. Furthermore, a measuring system must automati-

cally detect in good time contaminations in hoses, apparatuses and reactors in order to be able to trigger a cleaning process.

Preferably, all the apparatuses, pumps and shafts used are connected to the respective module control computer of the module, which module control computer reports faults to the central control computer. Depending on the type of fault, measures are initiated either by the central control computer or by the respective module control computer in order to eliminate the fault.

The invention makes provision for the cell cultures in the bioreactors to be checked preferably recurrently during operation, for example on a daily basis, for contaminations, i.e. in particular measurement of optical density, pH, spectral analysis and glucose concentration, and proliferation TEER value measurement for determining the cell density in the bioreactor. In the case of contamination or in the absence of cell growth, bioreactors can in this way be removed in a targeted manner from the production process and be ejected from the plant. Prompt detection minimises the risk of cross-contaminations and allows a resource-sparing process sequence.

In order to define process parameters, the cell yields as expressed in particular as cell count and vitality are determined, preferably by use of an automated cell counting apparatus, preferably after the cell isolation (cell extraction module) and cell expansion (cell expansion module). A small sample amount is removed using a pipette and supplied to the cell counting apparatus via a hose system. After the measurement has been carried out, the result is transmitted to the central control computer and/or the respective module computer.

The invention is illustrated in greater detail in the figures which do not, however, entail any limitation. In the drawings:

Figure 1 is a schematic overview of the arrangement of the three modules according to the invention, which are connected in series, within a clean room. The maintenance access, which is shown to be transparent, is arranged in the foreground. The material locks for media and tissues are arranged in the upper half of the rear wall; the accesses for the disposal of waste are located in the lower half of the rear wall, below the perforated intermediate bottom.

Figure 2 is a schematic overview of the basic construction of the modules according to the invention which are connected in series. The module 1 according to the invention has at least one incubator, a pipetting station, a device for fat separation and a device for separating tissue, in particular chopper. The biopsate introduced via the input interface is brought to the various processing stations via the handling device, which is preferably a module-overlapping automatic pipette, in accordance with the course of the process and is subsequently transferred, in the form of isolated cells, to the second module at the first transfer interface.

The second module has at least one media exchange station, a pipetting station, an incubator, a measuring station for cell culture parameters, such as inter alia pH oxygen partial pressure, media opacity and TEER value, and also a cell counting station. The isolated cells transferred from the first transfer interface are brought to the individual stations via a handling device, which is preferably a robot system, in accordance with the process sequence. Over the course of the sequence the isolated cells are metered to an empty reactor for cultivating the cells, which is preferably introduced into the module via a lock, seeded in a metered manner and incubated. The cells which are propagated as a result form a cell cluster or a cell suspension which is transferred to the third module via the second transfer interface.

The third module has at least one dispensing station, an incubator, a decapper and, if appropriate, an OCT measuring station. The propagated cells transferred from the second module via the second transfer interface (in the form of a cell suspension or a cell cluster) are transferred to the individual stations via a handling system, which is in particular a robot system, in accordance with the process sequence according to the invention. After the sequential build-up of tissue the newly constituted tissues constructs are, if appropriate, checked in an OCT measuring station for their construction and thus for quality. Subsequently, the tissue is ejected from the system, preferably directly out of the incubator which preferably serves to intermediately store the newly constituted tissues, via the output interface of the third module.

Figure 3 shows schematically the architecture of the control computers, the data connections and the preferably transmitted states and control conditions against the background of the controlling of the flow of media from biopsate via isolated primary cells, via cultivated cells up to the finished newly constituted tissue.

Figure 4 is a schematic overview of the flows of media in the interconnected modules according to the invention. Input variables designate the materials introduced into the respective modules via the media locks; the output variables designate substantially the waste substances which are generated in the respective modules and are to be ejected.

Claims

1. Method for the automatic production of biological tissue newly constituted from animal or human donor tissue, including the steps:

- providing the donor tissue at an input interface of a first module which is specifically embodied to isolate from the donor tissue donor tissue cells of at least one cell type and to single out these cells (cell extraction module), so as to obtain singled-out cells of the at least one cell type that are isolated from the donor tissue;

- transferring the isolated cells at a first interface of transition to a second module which is specifically embodied to propagate the respectively isolated cells of the at least one cell type (cell expansion module), so that in each case at least one cell group made up of propagated cells of the at least one cell type is obtained from the isolated cells;

- transferring the at least one cell group at a second interface of transition to a third module which is specifically embodied to newly constitute a biological tissue with one or more of the cell groups (tissue construction module), so that a newly constituted biological tissue is obtained from one or more cell groups of at least one respective cell type; and

- providing the newly constituted biological tissue at an output interface.

2. Method according to claim 1, wherein the process control within the modules is carried out by module control computers respectively associated with the modules.

3. Method according to claim 1 or 2, wherein the first module has between the input interface and first transition interface a first material flow rate, the second module has between the first transition interface and second transition interface a second material flow rate and the third module has between the second transition interface and output interface a third material flow rate and one or more of the first, second and third material flow rates is controlled as a function of at least one material flow rate different therefrom, selected from the first, second and third material flow rate, and/or of at least one state variable of at least one of the first, second and third modules in order to adapt the material flow rates of the modules.

4. Method according to claim 3, wherein the material flow rate is controlled by controlling the timing of the module.

5. Method according to claim 3 or 4, wherein the material flow rate is adapted by intermediate buffering of the material flow within a module.

6. Method according to one of claims 3 to 5, wherein the material flow rate in the first and/or second module is controlled as a function of the cell type processed in the third module.

7. Method according to one of claims 3 to 6, wherein the material flow rate in the first module is controlled as a function of the number of cells propagated in the second module.

8. Method according to one of claims 3 to 7, wherein the state variable is the cell count.

9. Method according to one of claims 3 to 8, wherein the state variable is the cell density.

10. Method according to one of claims 3 to 9, wherein the state variable is the cell vitality.

11. Method according to one of claims 3 to 10, wherein the state variable is the rate of proliferation of the cells.

12. Method according to one of the preceding claims, wherein the automatic isolation of the cells from the donor tissue in the first module is carried out in at least the following partial steps:

- automatic separating-off of fatty tissue which may be present on the donor tissue;

- automatic incision for breaking open the tissue structure of the donor tissue;

- automatic incubating of the donor tissue with the broken-open tissue structure in enzyme solution for separating the cell group of a first cell type;

- automatic separating-off of the individual cells detached from the cell group of the first cell type of the incubated donor tissue from the residual tissue of a further cell type; and

- automatic resuspending of the individual cells; so that a suspension of cells of a cell type that are isolated from the donor tissue is obtained.

13. Method according to claim 12 with the further partial steps:

- automatic incubating of the residual tissue in enzyme solution for breaking up the cell group of the residual tissue into individual cells of the further cell type,

- automatic resuspending of the cells detached from the cell cluster of the residual tissue.

14. Method according to one of the preceding claims, wherein the automatic propagation of isolated cells in the second module to form a cell group is carried out in at least the following partial steps:

- determining the cell count of the isolated cells based on a cell sample taken from the suspension of the isolated cells in a counting chamber,

- setting the cell count of the isolated cells in the suspension to a predefined cell concentration,

- seeding the isolated cells, if appropriate aliquoting the cells into a bioreactor,

- incubating the cells seeded on or in the bioreactor for cell propagation.

15. Method according to claim 14 with the further partial steps:

- singly or multiply determining cell cultivation parameters, selected from pH, oxygen partial pressure, TEER, glucose content, opacity, cell count and cell density, before, during or following the incubation.

16. Method according to one of the preceding claims, wherein the automatic build-up of tissue from at least one cell group of propagated cells in the third module is carried out in at least the following partial steps:

- automatic bringing of a tissue carrier into contact with a first layer of the cell group,

- incubating the layered build-up thus produced in order to constitute a newly constituted biological tissue.

17. Method according to claim 16 with the further partial step:

- applying a second or further layer of a cell group of propagated cells of a further cell type to the first layer.

18. Method according to claim 16 or 17 with the further partial step:

- determining the tissue quality.

19. Method according to claim 18, wherein the tissue quality is determined by means of optical coherence tomography (OCT).

20. Method according to one of the preceding claims, wherein the donor tissue is a biopsate of the human or animal body.

21. Method according to one of the preceding claims, wherein the donor tissue is multilayered skin tissue.

22. Method according to one of the preceding claims, wherein the cell type are fibroblasts.

23. Method according to one of the preceding claims, wherein the cell type are keratinocytes.

24. Method according to one of the preceding claims, wherein the newly constituted biological tissue is a multilayered tissue.

25. Method according to the preceding claim, wherein the newly constituted biological tissue contains at least one cell layer of fibroblasts and at least one cell layer of keratinocytes.

26. Artificial multilayered skin tissue, producible using the method according to one of the preceding claims.

27. Device for the automatic production of a biological tissue newly constituted from animal or human donor tissue, comprising:

- a first module (cell extraction module) which is specifically embodied to isolate from the donor tissue a donor tissue cell of at least one cell type and to single out these cells, comprising an input interface and a first transfer interface for transferring the isolated cells,

- a second module (cell expansion module), specifically embodied to propagate the isolated cells of the in each case at least one cell type, which is connected downstream of the first module and shares therewith the first transfer interface, comprising a second transfer interface for transferring a group of propagated cells; and

- a third module (tissue constitution module), specifically embodied to newly constitute a biological tissue with the at least one cell group, which is connected downstream of the second module and shares therewith the second transfer interface, comprising an output interface for ejecting the newly constituted biological tissue.

28. Device according to claim 27, also comprising a control unit for detecting material flow and/or state variables of at least one of the modules, wherein the unit is specifically embodied to control the material flow in at least one module as a function of the material flow and/or at least one state variable of at least one further module.

29. Device according to claim 27 and 28, wherein the first module comprises:

- a fat separator device for the separating-off of fatty tissue which may be present on the donor tissue from the donor tissue,

- a chopper device for cutting into the tissue structure of the donor tissue,

- an incubator for incubating the donor tissue with the cut-into tissue structure,

- a pipetting device for the separating-off of cells isolated from the donor tissue from the residual tissue.

30. Device according to one of claims 27 to 29, wherein the second module comprises:

- a pipetting device for holding the isolated cells in suspension and for metering the cell suspension into or onto a bioreactor,

- a cell counting device for determining the cell count in the cell suspension,

- an incubator for incubating the isolated cells seeded on or in a reactor.

31. Device according to claim 30, also comprising:

- a measuring station for determining the state parameters of the seeded cells cultivated in or on the bioreactor.

32. Method according to one of claims 27 to 31, wherein the third module has:

- a handling device for applying cultivated cell clusters to a tissue carrier,

- a measuring device for determining the tissue quality,

- an incubator for incubating the tissue carrier and the cell cluster attached thereto.

33. Device according to one of claims 27 to 32, wherein one or more of said modules each comprising at least one module control computer for respective controlling the processes in the module and, if appropriate, for determining the material flow rate and/or at least one state variable of the module.

34. Device according to claim 33, wherein the at least one module control computer is in data connection with a central control computer.

35. Computer program product for controlling the material flow in a device characterized in claims 27 to 34, where the first module has between the input interface and first transition interface a first material flow rate, the second module has between the first transition interface and second transition interface a second material flow rate and the third module has between the second transition interface and output interface a third material flow rate, characterized by the program steps:

- detecting one or more of the first, second and third material flow rates and/or one or more state variables of the first, second and third modules;

- controlling at least one first, second and third material flow rate different therefrom, as a function of the detected at least one material flow

rate and/or state variable in order to adapt the material flow rates of the modules to one another.

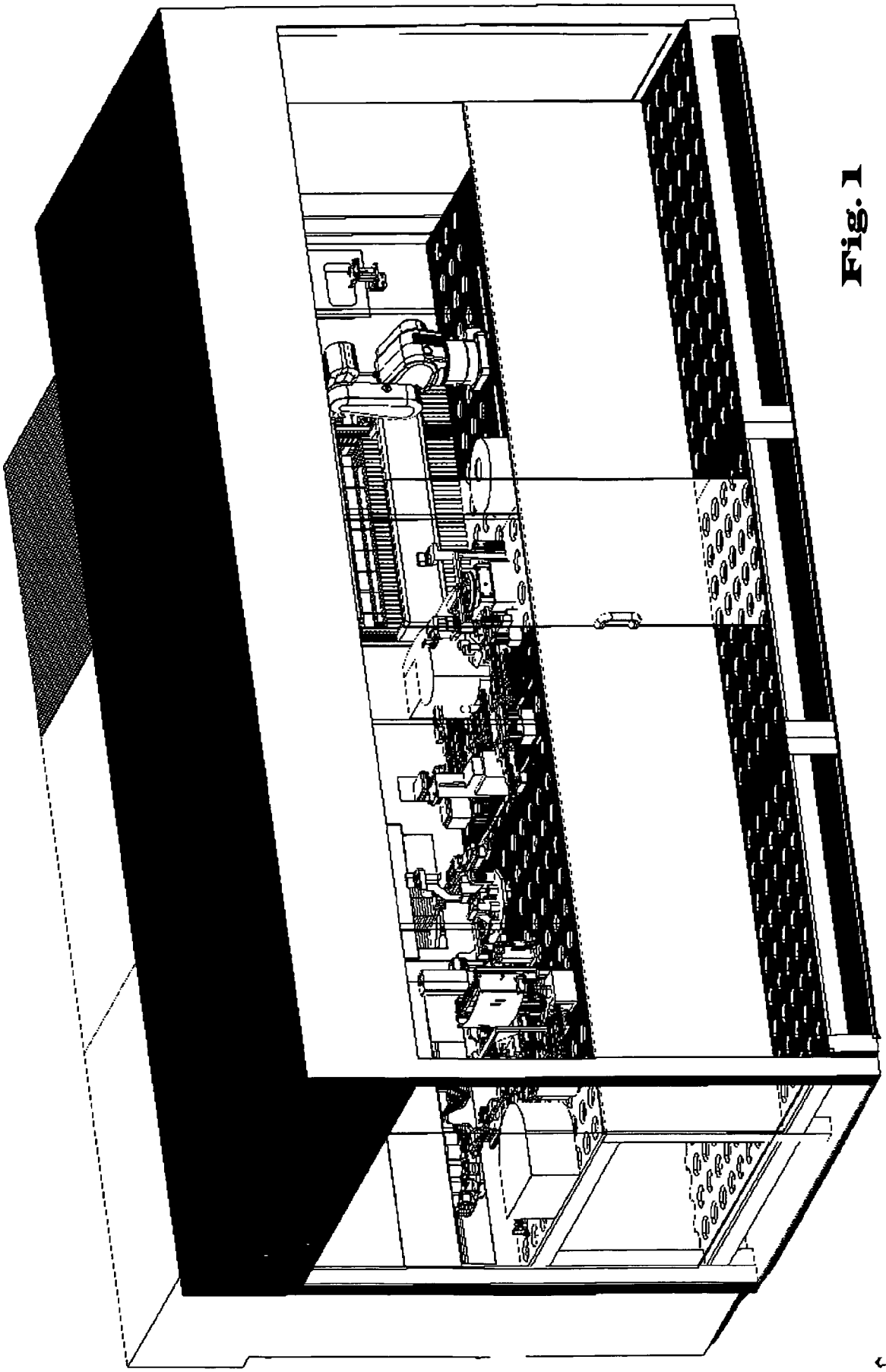

Fig. 1

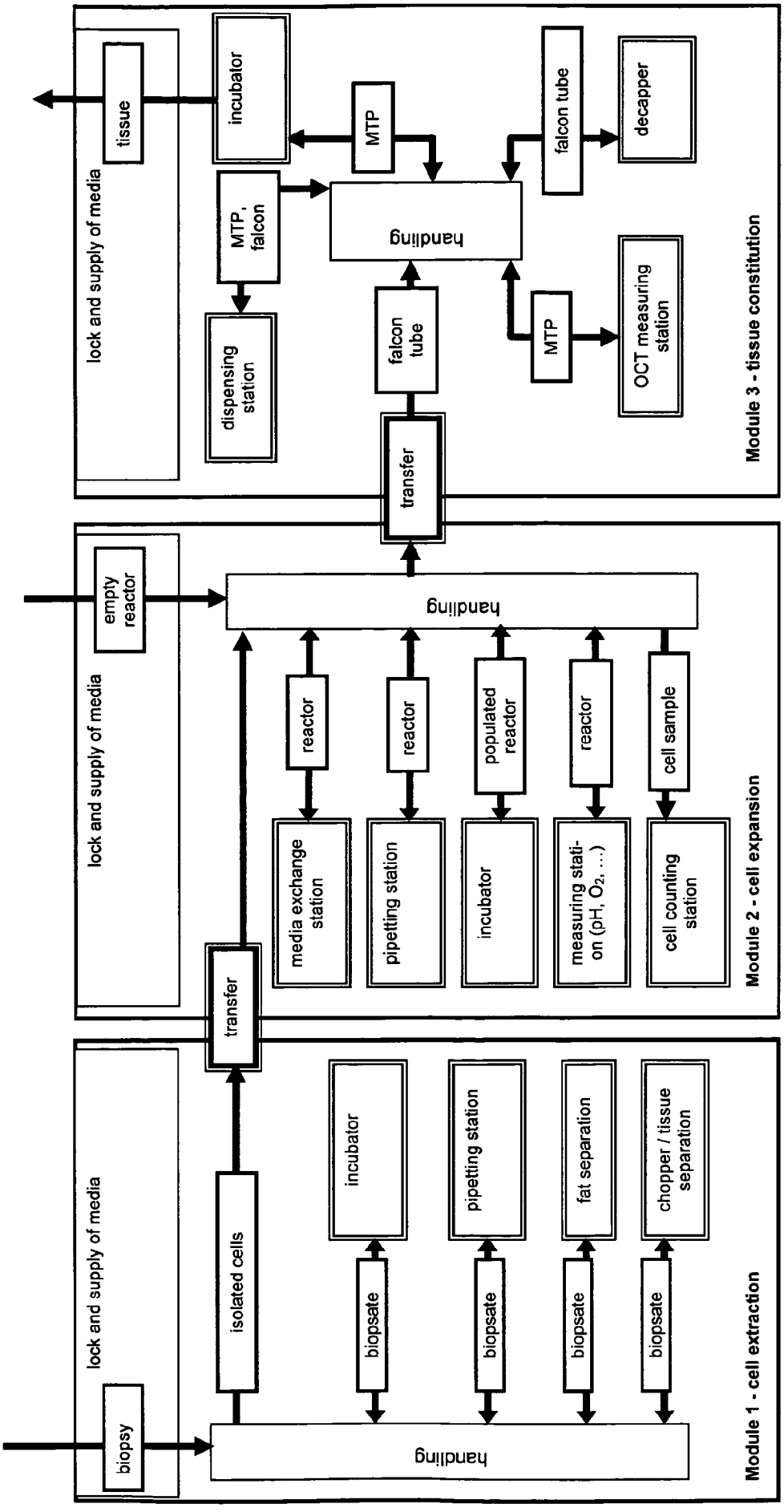
Module 1 - cell extraction
lock and supply of media
biopsy
handling
isolated cells
biopsate
incubator
biopsate
pipetting station
biopsate
fat separation
biopsate
chopper / tissue separation
transfer
Module 2 - cell expansion
lock and supply of media
empty reactor
handling
reactor
media exchange station
reactor
pipetting station
populated reactor
incubator
reactor
measuring station ($pH, O_2, ...$)
cell sample
cell counting station
transfer
Module 3 - tissue constitution
lock and supply of media
tissue
incubator
MTP
falcon tube
decapper
MTP, falcon
handling
dispensing station
falcon tube
MTP
OCT measuring station
transfer

Fig. 2

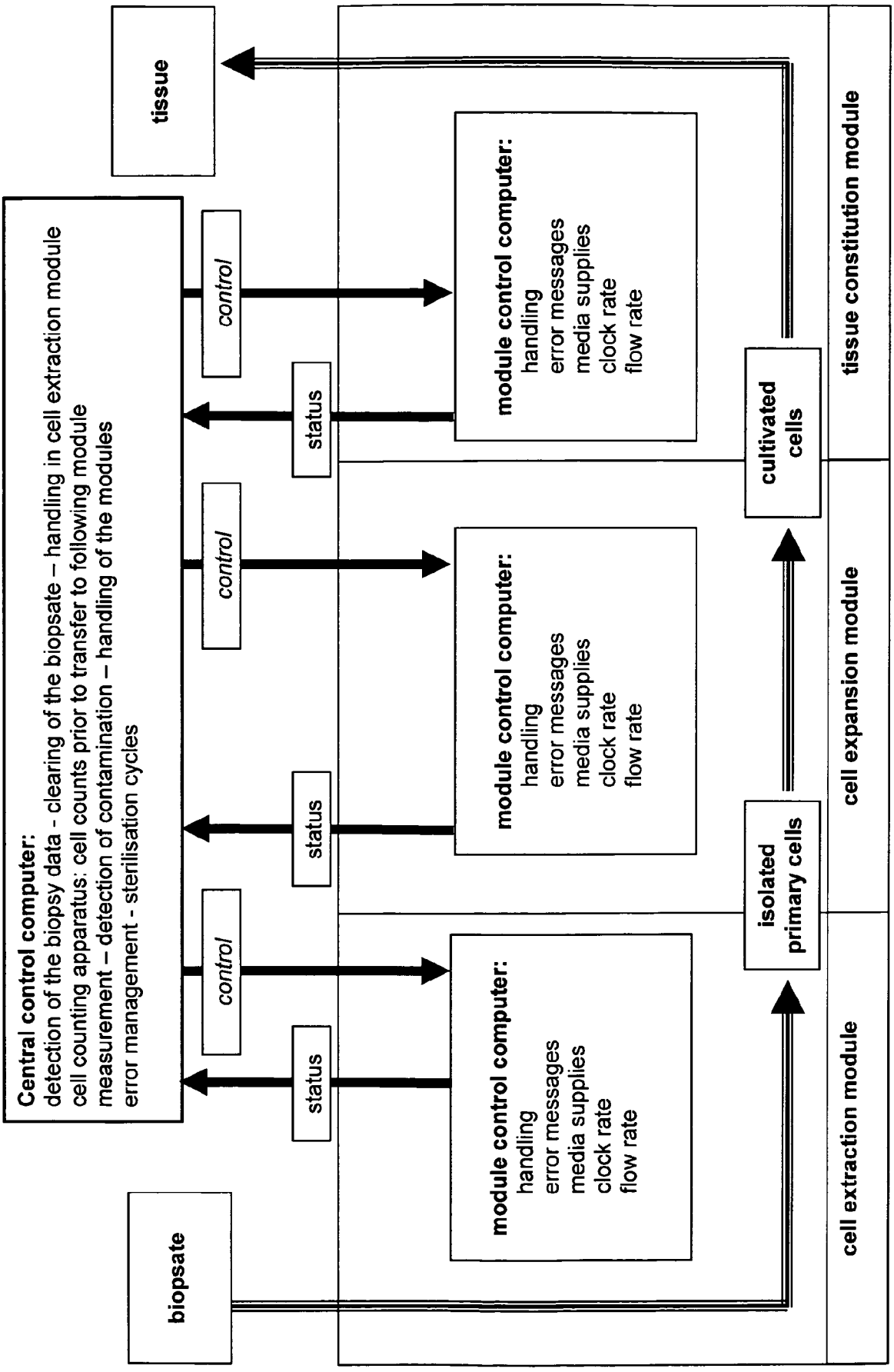
tissue
Central control computer:
detection of the biopsy data - clearing of the biopsate – handling in cell extraction module
cell counting apparatus: cell counts prior to transfer to following module
measurement – detection of contamination – handling of the modules
error management - sterilisation cycles
control
status
module control computer:
handling
error messages
media supplies
clock rate
flow rate
cultivated cells
tissue constitution module
isolated primary cells
cell expansion module
biopsate
cell extraction module

Fig. 3

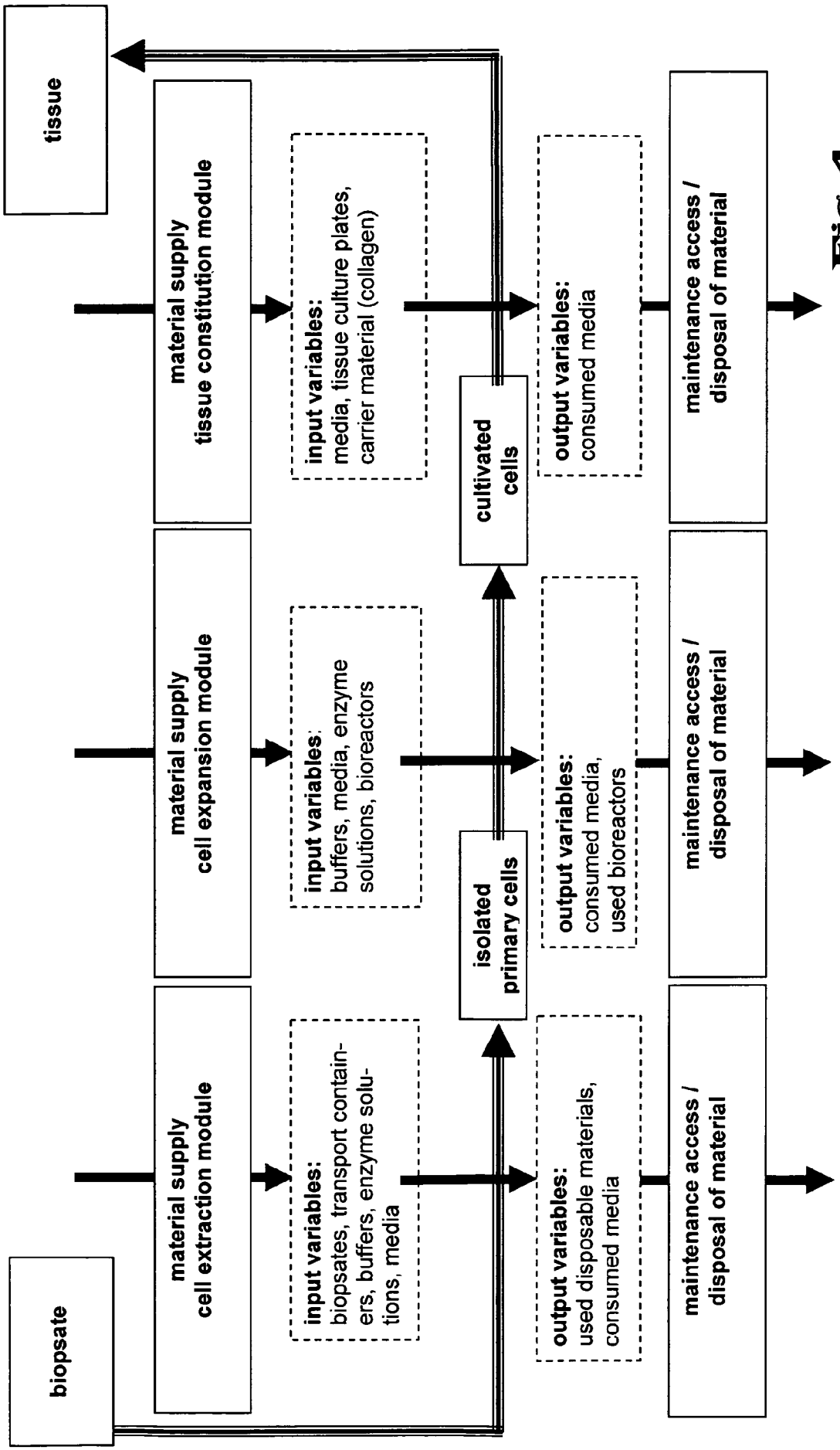
biopsate
material supply
cell extraction module
input variables:
biopsates, transport containers, buffers, enzyme solutions, media
output variables:
used disposable materials, consumed media
maintenance access / disposal of material
isolated primary cells
material supply
cell expansion module
input variables:
buffers, media, enzyme solutions, bioreactors
output variables:
consumed media, used bioreactors
maintenance access / disposal of material
cultivated cells
material supply
tissue constitution module
input variables:
media, tissue culture plates, carrier material (collagen)
output variables:
consumed media
maintenance access / disposal of material
tissue

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/001076

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M3/00 A61L27/60 C12N5/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12M A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2006/141623 A1 (SMITH ET AL.) 29 June 2006 (2006-06-29) paragraphs [0001], [0004], [0005], [0010] - [0029], [0044] - [0066], [0084], [0085], [0111] - [0119] paragraphs [0144], [0153] - [0171], [0176], [0178], [0180] - [0184], [0187], [0188], [0193], [0195], [0196] paragraphs [0198], [0199], [0216] - [0220] figures 1-23	1-25,27, 28,30-35 29
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Y	US 2008/014181 A1 (ARIFF ET AL.) 17 January 2008 (2008-01-17) paragraph [0046]	29

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2010	24/08/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Alvarez Alvarez, C

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/001076

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