

TITLE OF INVENTION: [TISSUE DISSOCIATION SYSTEM AND TISSUE DISSOCIATION METHOD]

PRIORITY APPLICATIONS

[0001] The present application claims priority to the Chinese invention patent application (Application No. 2023112451186) titled "Tissue Dissociation System and Tissue Dissociation Method" filed on September 25, 2023, the entire disclosure of which is incorporated herein by reference.

TECHNICAL FIELD

[0002] The present application belongs to the technical field of primary cell separation, and particularly relates to a tissue dissociation system and a tissue dissociation method.

BACKGROUND

[0003] Primary cells are cells directly isolated and cultured from tissues or organs in the body, and primary cells also have wide applications in clinical medicine and treatment. For example, tumor-infiltrating lymphocytes (TILs) are isolated from tumor tissue, and TILs refer to lymphocytes present in tumor tissue, tumor metastatic lymph nodes, and other tissues. TIL cells contain a relatively high proportion of tumor-specific lymphocytes, and this type of lymphocyte, after in vitro isolation, amplification, and activation, has already been used as a clinical treatment method for adoptive cellular immunotherapy of tumors, and has achieved relatively good clinical results in the treatment of cancers such as malignant melanoma.

[0004] The traditional tissue dissociation method is to transfer specimens obtained clinically (for example, from a sterile operating room) to a laboratory, cut the tissue mass with sterile scissors on a clean bench, then chop into small pieces with scissors or a blade, allowing cells to freely disperse, followed by subsequent culture and amplification. This method can never avoid the risk of exposing the tissue mass, with problems of susceptibility to contamination and low efficiency. As a result, the production of this type of cell therapy product requires a high level of environmental cleanliness, which increases cost.

[0005] To avoid contamination, it is usually necessary to use a closed container to store the tissue sample obtained from a sterile environment (such as an operating room), and transfer it to a laboratory, and then under another sterile operating environment (such as under large sterile operating equipment), add the tissue sample into a tissue dissociation device for dissociation, and after dissociation, transfer it into a culture dish in the sterile operating environment. However, this approach requires separate sterile operating environments, which greatly increases cost, and not only makes the entire operating process relatively complicated, but also places high demands on personnel; moreover, for some remote areas, or some laboratories, testing institutions, or medical institutions with relatively poor conditions, because they cannot purchase large sterile operating equipment or provide a sterile operating environment, they are unable to carry out tissue dissociation, and therefore unable to carry out tissue culture.

SUMMARY

[0006] The objective of the present application is to provide a tissue dissociation system and a tissue dissociation method, by enabling tissue dissociation, transfer, and culture to be carried out in a fully sealed environment throughout the entire process, reducing the risk of tissue being contaminated during dissociation and transfer.

[0007] To solve the aforementioned technical issues, the present application

specifically adopts the following technical solutions: A first aspect of the present application is to provide tissue dissociation system, which comprises: a tissue dissociator, a culture fluid bag, a sterile culture bag, and a tube connecting machine used to separately connect an outlet tube of the culture fluid bag and an inlet tube of the sterile culture bag with the tissue dissociator; in an initial state, free ends of the outlet tube and the inlet tube (ends not connected to the culture fluid bag and the sterile culture bag) are in a sealed state.

[0008] Wherein, the tissue dissociator comprises: a sliding sleeve, a base detachably connected to one end of the sliding sleeve, and a dissociation piston rod axially movable to the base within an inner space of the sliding sleeve; when the base is connected with the sliding sleeve, the base and the axial inner space of the sliding sleeve form a dissociation chamber for providing an axial movement path for the dissociation piston rod, an end of the dissociation piston rod close to the base being provided with a dissociation portion for dissociating tissue; the dissociation chamber is connected to an inlet pipe and an outlet pipe, ends of the inlet pipe and outlet pipe not connected to the dissociation chamber (otherwise referred to as free ends) being sealed.

[0009] In some embodiments of the present application, the inlet pipe and the outlet pipe are symmetrically arranged on two sides of the dissociation chamber, and the inlet pipe and the outlet pipe are arranged slanted, so that an extension line of a respective centerline of each of the inlet pipe and the outlet pipe passes through the bottom of the dissociation chamber.

[0010] In some embodiments of the present application, a return spring is sleeved on the dissociation piston rod; one end of the return spring abuts against the dissociation piston rod, and the other end abuts against a spring seat in the sliding sleeve.

[0011] In some embodiments of the present application, an end of the dissociation piston rod is arranged with a dissociation piston, and the dissociation portion is mounted at an end of the dissociation piston; the diameter of the dissociation piston is larger than the diameter of the dissociation piston rod.

[0012] In some embodiments of the present application, the upper end of the dissociation chamber is arranged with a limit step.

[0013] In some embodiments of the present application, an annular sealing ring is arranged on the dissociation piston rod; and/or, the upper end of the dissociation piston rod extends out of the sliding sleeve and is arranged with a pressing disc.

[0014] In some embodiments of the present application, the sliding sleeve comprises detachable upper and lower sections, and the dissociation piston rod comprises detachable upper and lower sections; the lower dissociation piston rod is sleeved with an elastic sealing sleeve.

[0015] In some embodiments of the present application, the culture fluid bag comprises a first chamber and a second chamber; the first chamber is connected to an end of the outlet tube; a partition film is arranged between the first chamber and the second chamber; the partition film can be opened under external force, so that the first chamber and the second chamber are connected; wherein, the first chamber pre-stores a second preset volume of culture fluid less than a third preset volume of culture fluid stored in the second chamber.

[0016] In some embodiments of the present application, the sealing sleeve is in the shape of an inverted bowl, with the large end connected inside the sliding sleeve, and the small end connected on the lower dissociation piston rod, so that a connection portion between the dissociation piston rod and the sliding sleeve is sealed.

[0017] A second aspect of the present application is to provide a tissue dissociation method based on the aforementioned tissue dissociation system, comprising the steps: separating the base and the sliding sleeve, which were pre-placed in a sterile collection

environment, and placing obtained tissue to be dissociated into the base, and then connecting the base and the sliding sleeve, so that a dissociation chamber is formed between the base and the axial inner space of the sliding sleeve for providing an axial movement path for the dissociation piston; using the tube connecting machine to connect the culture fluid bag and the sterile culture bag respectively to the inlet pipe and the outlet pipe of the dissociation chamber, so that the culture fluid bag and the sterile culture bag are connected with the dissociation chamber; squeezing the culture fluid bag, causing the second preset volume of culture fluid stored in the culture fluid bag to enter the dissociation chamber through the inlet pipe; driving the dissociation piston rod to dissociate the tissue to be dissociated, such that during the entire dissociation process, an outlet path between the sterile culture bag and the dissociation chamber remains unobstructed; after dissociation is completed, squeezing the culture fluid bag to cause the remaining culture fluid in the culture fluid bag to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag; blocking the outlet passage between the sterile culture bag and the dissociation chamber, and separating the sterile culture bag from the tissue dissociator, such that the inlet tube of the sterile culture bag after separation is in a sealed state.

[0018] In some embodiments of the present application, the step of squeezing the culture fluid bag to cause the second preset volume of culture fluid to enter the dissociation chamber specifically comprises the following steps: squeezing the first chamber of the culture fluid bag in the direction of the outlet tube on the culture fluid bag, causing the second preset volume of culture fluid stored in the first chamber to enter the dissociation chamber through the outlet tube.

[0019] In some embodiments of the present application, after dissociation is completed, the steps of squeezing the culture fluid bag to cause the remaining culture fluid in the culture fluid bag to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag specifically comprises the following steps: squeezing the second chamber of the culture fluid bag, causing the partition film between the first chamber and the second chamber to open, thereby causing the third preset volume of culture fluid prestored in the second chamber to enter the first chamber; repeatedly squeezing the first chamber in the direction of the outlet tube, causing the third preset volume of culture fluid to enter the dissociation chamber through the outlet tube to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag.

[0020] In some embodiments of the present application, the steps of repeatedly squeezing the first chamber along the direction of the outlet tube specifically comprises: squeezing the first chamber to cause the culture fluid to enter the dissociation chamber; oscillating or shaking the tissue dissociator, causing most of the tissue particles attached to the chamber wall of the dissociation chamber to detach from the chamber wall and gather at the bottom of the dissociation chamber; placing the tissue dissociator at an angle and squeezing the first chamber repeatedly, causing the culture fluid under the action of squeezing and gravity to flush the bottom of the dissociation chamber, thereby flushing the dissociated tissue particles into the sterile culture bag.

[0021] The advantageous aspects of the present application are: a portion of the tissue dissociator in the present application, such as the base or the base and a portion of the sliding sleeve (see embodiments), can be used as single-use consumables; during use, the base can receive a specimen obtained clinically (tissue to be dissociated) in a sterile environment and immediately connect with the sliding sleeve to form a seal; then, the tube connecting machine connects the culture fluid bag and the sterile culture bag respectively with the originally sealed inlet pipe and outlet pipe; then, the dissociation operation is performed in the dissociation chamber formed by the base and the sliding sleeve; when tissue dissociation is completed, the culture fluid in the culture fluid bag

flushes the dissociation chamber to flush the dissociated tissue into the sterile culture bag to complete the transfer of tissue, and after transfer, a heat sealer or other measures can be used to close/block the outlet passage between the sterile culture bag and the dissociation chamber while separating the sterile culture bag from the tissue dissociator, and the tube of the separated sterile culture bag remains in a closed state, enabling sterile culture to be carried out directly in the sterile culture bag, without the need to separately provide a closed container for culture; the entire process of dissociation and transfer of tissue, and even the process of culture, are carried out in a sealed environment, without the need to separately provide a sterile environment, making tissue dissociation more convenient and reducing environmental constraints, while eliminating the risk of the dissociated tissue being contaminated.

[0022] The entire process of dissociation and transfer of tissue is carried out in a sealed environment, without the need to separately provide a sterile environment, making tissue dissociation more convenient and reducing environmental constraints, thereby greatly reducing the requirements for operators, reducing costs, and enabling even remote areas or laboratories, medical institutions, and testing institutions with poor economic conditions to carry out tissue dissociation and culture, which has higher flexibility and broader adaptability compared with traditional tissue dissociation devices.

[0023] Furthermore, in the present application, a certain amount of sterile gas is pre-stored in the culture bag (of course, preferably, the volume of gas in the sterile culture bag is less than the capacity of the sterile culture bag; specifically, its volume only needs to allow the dissociation piston to move freely within the activity space defined by the sliding sleeve and the dissociation chamber), so that in a sealed environment, the operation stroke of the dissociation piston is prevented from being limited due to insufficient pressure in the sealed space caused by misoperation or other reasons.

[0024] Still further, according to different operation processes, such as the dissociation process and the transfer process requiring different amounts of culture fluid, the present application stores a certain amount in each of the two chambers of the culture fluid bag, thereby avoiding too much culture fluid being squeezed into the dissociation chamber during the dissociation process due to unstable manual operation, which weakens the force of the dissociation piston on the tissue, resulting in longer dissociation operating times, or even overflow of culture fluid; or too little, causing the force of the dissociation piston directly acting on the tissue to be too strong, which damages the tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] In order to more clearly describe the technical solutions of the embodiments of the present application or of the prior art, the drawings that need to be used in the descriptions of the embodiments or the prior art are briefly introduced below. In all drawings, similar elements or parts are generally identified by similar reference numerals. In the drawings, each element or part is not necessarily drawn according to actual proportions. It is obvious that the drawings in the following descriptions are some embodiments of the present application, and for those of ordinary skill in the art, other drawings can also be obtained according to these drawings without creative effort.

[0026] FIGURE 1 is a front view of a tissue dissociator in a tissue dissociation system of one exemplary embodiment of the present application.

[0027] FIGURE 2 is a cross-sectional view of the tissue dissociator in the tissue dissociation system of one exemplary embodiment of the present application.

[0028] FIGURE 3 is a perspective view of the tissue dissociator in the tissue dissociation system of one exemplary embodiment of the present application.

[0029] FIGURE 4 is a front view of the tissue dissociator in the tissue dissociation system of another exemplary embodiment of the present application.

[0030] FIGURE 5 is a cross-sectional view of the tissue dissociator in the tissue dissociation system of another exemplary embodiment of the present application.

[0031] FIGURE 6 is a front view of the tissue dissociator in the tissue dissociation system of yet another exemplary embodiment of the present application.

[0032] FIGURE 7 is a cross-sectional view of the tissue dissociator in the tissue dissociation system of yet another exemplary embodiment of the present application.

[0033] FIGURE 8 is a schematic diagram showing the cooperation among the tissue dissociator, a culture fluid bag, and a sterile culture bag in the tissue dissociation system of one exemplary embodiment of the present application.

[0034] FIGURE 9 is a schematic flow chart of a tissue dissociation method of one exemplary embodiment of the present application.

[0035] Reference numbers in the drawings: 1 sliding sleeve, 2 dissociation piston rod, 3 base, 4 inlet pipe, 5 outlet pipe, 6 pressing disc, 7 dissociation piston, 8 dissociation chamber, 81 piston activity space, 9 annular sealing ring, 10 return spring, 11 sealing sleeve, 101 upper sliding sleeve, 102 lower sliding sleeve, 201 upper dissociation piston rod, 202 lower dissociation piston rod, 21 first chamber, 22 second chamber, 23 outlet tube, 24 partition film, 31 inlet tube.

DETAILED DESCRIPTION OF EMBODIMENTS

[0036] To make the objectives, technical solutions, and advantages of the embodiments of the present application clearer, the following in combination with the drawings in the embodiments of the present application will clearly and completely describe the technical solutions in the embodiments of the present application. Of course, the described embodiments are part of the embodiments of the present application and not all of the embodiments. Based on the embodiments of the present application, all other embodiments obtained by those of ordinary skill in the art without creative work shall fall within the protection scope of the present application.

[0037] In this document, suffixes such as “module,” “part,” or “unit” used to denote elements are only for facilitating the description of the present application and have no specific meaning in themselves. Therefore, “module,” “part,” or “unit” can be used interchangeably.

[0038] In this document, terms such as “upper,” “lower,” “inner,” “outer,” “front,” “rear,” “one end,” and “the other end” indicating orientation or positional relationships are based on the orientation or positional relationships shown in the drawings, and are only for convenience in describing the present application and simplifying the description, rather than indicating or implying that the device or element referred to must have a specific orientation, be constructed, or operate in a specific orientation, and thus cannot be understood as limiting the present application. In addition, the terms “first” and “second” are only used for descriptive purposes and cannot be understood as indicating or implying relative importance.

[0039] In this document, unless expressly specified and defined otherwise, terms such as “mounted,” “provided with,” and “connected” shall be understood broadly. For example, “connected” may be fixedly connected, or may be detachably connected, or integrally connected; they may be mechanically connected, or directly connected, or may be indirectly connected through an intermediate medium; there may be communication between the interiors of two elements. For those of ordinary skill in the art, the specific meaning of the above terms in the present application can be understood according to the specific situation.

[0040] In this document, “and/or” includes any and all combinations of one or more of the listed related items. In this document, “multiple” means two or more, meaning it includes two, three, four, five, and so on.

[0041] In this document, “sterile environment” or “sterile collection environment” refers to a clean operating environment with the highest degree of cleanliness, such as an operating room. In this document, “dissociation operating environment” refers to various clean operating environments not with the highest degree of cleanliness (such as a clean operating area of a testing room), or uncleaned operating environments (such as offices of medical institutions or testing institutions).

[0042] Chinese Patent CN201880042732.3 discloses a tissue dissociator including a blade holder, the blade holder having a blade; and a sample holder, the sample holder including a tissue actuator having a distal flexible stop, wherein the tissue actuator is configured to be displaced along a longitudinal axis within the sample holder. The prior art mentioned above only proposes solutions for dissociation of tissue and does not include methods for ensuring that the tissue is not contaminated during dissociation and transfer. In practice, the above-described techniques also require operation within a sterile room.

[0043] In view of the above problems, the present application provides a tissue dissociator, and further provides a tissue dissociation system including the tissue dissociator, and provides a tissue dissociation method based on the tissue dissociation system.

[0044] EMBODIMENT 1:

[0045] As shown in FIGURES 1-3, the tissue dissociator in the present embodiment includes a sliding sleeve 1, a base 3 detachably connected to one end of the sliding sleeve 1, and a dissociation piston rod 2 axially movable within an inner space of the sliding sleeve 1, the end of the dissociation piston rod 2 close to the base 3 being provided with a dissociation portion for dissociating tissue. Wherein, the base 3 is internally arranged with a dissociation chamber 8 with an open top, the dissociation chamber 8 communicating with an axial inner cavity of the sliding sleeve 1, and the end of the axial inner cavity of the sliding sleeve 1 close to the base 3 is arranged with a mounting groove matched with the base. When the base 3 is connected with the sliding sleeve 1, the top of the mounting groove cooperates with the opening of the dissociation chamber 8, thereby forming a dissociation chamber for providing an axial movement path for the dissociation piston rod 2. Specifically, a dissociation piston 7 with blade provided on the dissociation piston rod 2 has a maximum stroke in the dissociation operating space that is the difference between the maximum height of the dissociation chamber 8 and the height of the dissociation piston 7 itself. The dissociation chamber 8 (that is, the dissociation chamber) is symmetrically connected with an inlet pipe 4 and an outlet pipe 5, and the ends of the inlet pipe 4 and the outlet pipe 5 not connected with the dissociation chamber 8 are in a sealed state.

[0046] In the present application, the objective of having the ends of the inlet pipe 4 and outlet pipe 5 not connected with the dissociation chamber 8 be sealed is to create a closed environment. After connecting the inlet pipe 4 and outlet pipe 5 respectively with a culture fluid bag and a sterile culture bag, the culture fluid bag and the sterile culture bag still communicate with the dissociation chamber (that is, the dissociation chamber 8) in a closed environment after connection. To achieve this objective, in the present embodiment, the inlet pipe 4 and the outlet pipe 5 are plastic tubes (such as PVC tubes), which achieve communication after being connected with the culture fluid bag and the sterile culture bag by a tube connecting machine.

[0047] Of course, it can be foreseen that the culture fluid bag and the sterile culture bag should also be provided with a plastic (such as PVC) connector. For example, the culture fluid bag arranged with a plastic tube (such as PVC tube) serves as an outlet tube, and the sterile culture bag arranged with a plastic tube (such as PVC tube) serves as an inlet

tube, for connecting with the inlet pipe 4 and the outlet pipe 5 under the action of the tube connecting machine.

[0048] In the present application, the tube joining machine is a medical device that combines two pipelines (for example, the inlet pipe 4 on the dissociation chamber 8 and an outlet tube 23 on the culture fluid bag; or the outlet pipe 5 on the dissociation chamber 8 and an inlet tube 31 on the sterile culture bag) in the dissociation operating environment (for example, an environment with a lower degree of cleanliness), and keeps the pipelines always in communication, mainly using the principle of heat-sealing to connect the two pipelines, which is indispensable in fields such as blood collection and supply, clinical surgery, and biopharmaceuticals.

[0049] In the present embodiment, the sliding sleeve 1 and the base 3 are detachably connected, and the connection method includes, but is not limited to, threads, buckles, interference fit, and the like, with the two connected together during dissociation, and the two separating to facilitate the placement and retrieval of tissue.

[0050] Since it is necessary to squeeze the culture fluid bag after dissociation to flush the dissociation chamber with culture fluid under certain pressure and carry the dissociated tissue into the sterile culture bag, the inlet pipe 4 and outlet pipe 5 are respectively arranged on two sides of the dissociation chamber 8. The inlet pipe 4 and the outlet pipe 5 are arranged slanted, and extension lines of their respective centerlines pass through the bottom of the dissociation chamber 8. In this way the continuity of pressure can be maintained, making the tissue in the entire dissociation chamber 8 flushed more cleanly.

[0051] In the present embodiment, an end of the dissociation piston rod 2 is arranged with the dissociation piston 7, and the dissociation portion is mounted at an end of the dissociation piston 7. The diameter of the dissociation piston 7 is larger than the diameter of the dissociation piston rod 2. The upper end of the dissociation chamber 8 is arranged with a limit step to prevent the dissociation piston rod 2 from slipping out of the sliding sleeve 1.

[0052] In order to prevent tissue from overflowing from the dissociation chamber 8 during dissociation, an annular sealing ring 9 can also be arranged on the dissociation piston rod 2 or the dissociation piston 7. Similarly, the annular sealing ring 9 can also prevent external impurities from entering the dissociation chamber and contaminating the tissue.

[0053] In some embodiments, the dissociation portion may be in the form of a blade array, but of course may also be in other forms. For example, grooves or protrusions may be provided at the end of the dissociation piston 7, making it unsmooth and uneven, so that in the reciprocating motion of the dissociation piston rod 2, the grooves or protrusions dissociate the tissue to be dissociated.

[0054] In order to facilitate driving the dissociation piston rod 2 to move, the upper end of the dissociation piston rod 2 extends out of the sliding sleeve 1 and is arranged with a pressing disc 6. The shape of the pressing disc 6 facilitates an operator to hold to push and pull the dissociation piston rod.

[0055] EMBODIMENT 2:

[0056] As shown in FIGURES 4 and 5, the differences from Embodiment 1 are:

[0057] In the present embodiment, a return spring 10 is sleeved on the dissociation piston rod 2. One end of the return spring 10 abuts against the dissociation piston rod 2, and the other end abuts against a spring seat in the sliding sleeve 1. Correspondingly, the top end of the sliding sleeve 1 is arranged with an accommodating space for accommodating the return spring 10.

[0058] In the present embodiment, the end of the axial inner cavity of the sliding sleeve

1 close to the base 3 is arranged as a piston activity space 81 that provides a portion of the movement path for the dissociation piston 7, which together with the dissociation chamber 8 of the base 3 (the cavity wall height of which is much smaller than that in Embodiment 1) forms the dissociation chamber. That is, the maximum stroke of the axial movement of the dissociation piston 7 is the sum of the height of the piston activity space 81 and the height of the cavity wall of the dissociation chamber 8 on the base 3 minus the height of the dissociation piston 7 itself. In addition, the inlet pipe 4 and outlet pipe 5 are symmetrically arranged on both sides of the piston activity space 81. When pushing the dissociation piston rod 2, the return spring 10 is compressed, and when the return spring 10 rebounds, it can reset the dissociation piston rod 2, thereby facilitating operation.

[0059] Specifically, referring to FIGURE 5, the axial inner cavity of the sliding sleeve 1 includes three sections. The first section serves as an accommodating cavity for the spring. The second section is matched with the dissociation piston rod 2. The third section serves as the piston activity space 81. Due to the arrangement of the spring accommodating cavity and the piston activity space 81, compared with the matching method between the dissociation piston rod 2 and the sliding sleeve 1 in Embodiment 1, the portion directly contacting the axial inner cavity of the sliding sleeve 1 is reduced, greatly reducing the friction force during the process of pushing the dissociation piston rod 2.

[0060] Preferably, the piston activity space 81 has a cylindrical cross-section and has a diameter larger than the diameter of the axial inner cavity corresponding to the dissociation piston rod 2 above it.

[0061] EMBODIMENT 3:

[0062] As shown in FIGURES 6 and 7, the differences from Embodiments 1 and 2 are:

[0063] In the present embodiment, the sliding sleeve 2 comprises detachable upper and lower sections, namely an upper sliding sleeve 101 and a lower sliding sleeve 102. In addition, the dissociation piston rod 2 comprises detachable upper and lower sections, namely an upper dissociation piston rod 201 and a lower dissociation piston rod 202.

[0064] Before dissociation, the lower sliding sleeve 102, the lower dissociation piston rod 202, and the base 3 can be separately placed as consumables in a sterile collection environment, and then assembled together to receive and seal the collected tissue to be dissociated, so that after long-distance transportation to a destination, they can then be assembled with the upper dissociation piston rod 201 and the upper sliding sleeve 101. Then, the dissociation operation is carried out.

[0065] After dissociation is completed, the lower sliding sleeve 102 remains connected to the base 3, and the lower dissociation piston rod 202 serves as a filler to close the cavity of the lower sliding sleeve 102. In this way a closed sterile environment can be maintained, suitable for long-distance transportation and then transfer through the culture fluid bag and the sterile culture bag.

[0066] In addition, in order to further improve sealing, in the present embodiment, an elastic sealing sleeve 11 is sleeved on the lower dissociation piston rod 202. The sealing sleeve 11 is in the shape of an inverted bowl, with the large end connected inside the sliding sleeve 1, and the small end connected on the lower dissociation piston rod 202, so that the connection portion of the dissociation piston rod 2 and the sliding sleeve 1 is sealed.

[0067] EMBODIMENT 4:

[0068] Based on any one of the tissue dissociators in Embodiments 1-3 above, the present application further provides a tissue dissociation system, which includes any one

of the tissue dissociators of the above embodiments, a culture fluid bag, a sterile culture bag, and a tube connecting machine used to connect the outlet tube 23 of the culture fluid bag and the inlet tube 31 of the sterile culture bag with the inlet pipe 4 and the outlet pipe 5 of the tissue dissociator.

[0069] In the present embodiment, referring to FIGURE 8, the culture fluid bag includes a first chamber 21 and a second chamber 22, as well as an outlet tube 23 connected with the first chamber 21. A partition film 24 is arranged between the first chamber 21 and the second chamber 22. The partition film 24 can be opened under external force, so that the first chamber 21 and the second chamber 22 are connected. Wherein, the first chamber 21 pre-stores a second preset volume of culture fluid less than a third preset volume of culture fluid stored in the second chamber.

[0070] Furthermore, in some embodiments, the sterile culture bag pre-stores a first preset volume of sterile gas. By pre-storing a certain amount of sterile gas in the culture bag (of course, preferably, the gas volume in the sterile culture bag is less than the capacity of the sterile culture bag; specifically, the volume only needs to allow the dissociation piston to move freely within the activity space defined by the sliding sleeve and the dissociation chamber), it provides for the sealed environment in which insufficient air pressure caused by misoperation or other reasons may limit the operating stroke of the dissociation piston.

[0071] EMBODIMENT 5:

[0072] Referring to FIGURE 9, based on the above tissue dissociation system, the present application further provides a tissue dissociation method, including:

[0073] S1. Separating the base 3 and the sliding sleeve 1, which were pre-placed in a sterile collection environment, and placing obtained tissue to be dissociated into the base 3. In some embodiments, when the tissue dissociator of Embodiments 1 or 2 above is used, the base is a disposable consumable, while the sliding sleeve 1 and the dissociation piston rod 2 can be reused. Therefore, the base 3 can be equipped in a sterile environment for sampling, such as an operating room, and of course, it can be foreseen that the base 3 needs to be sealed in a sealed bag before use. After obtaining the tissue to be dissociated, the base 3 is taken out from the sealed bag, and the tissue to be dissociated is placed into the dissociation chamber 8 of the base 3.

[0074] In other embodiments, when the tissue dissociator of Embodiment 3 above is used, the base 3, the lower sliding sleeve 102, and the lower dissociation piston rod 202 are all disposable consumables (of course, the culture fluid bag and the sterile culture bag are also disposable consumables), and can be stored separately in a sterile collection environment, while the upper sliding sleeve 101 and the upper dissociation piston rod 201 can be placed in a testing room or laboratory for reuse, so that it is not necessary to ensure proportional configuration between the base 3, the lower sliding sleeve 102, and the lower dissociation piston rod 202 and the upper sliding sleeve 101 and the upper dissociation piston rod 201. That is, one set of the upper sliding sleeve 101 and the upper dissociation piston rod 201 can be used for assembling with multiple sets of the base 3, the lower sliding sleeve 102, and the lower dissociation piston rod 202 to perform tissue dissociation and transfer operations, further reducing cost.

[0075] S2. Connecting the base 3 and the sliding sleeve 1 in a sterile collection environment, so that a dissociation chamber is formed between the base 3 and the axial inner space of the sliding sleeve 1 for providing an axial movement path for the dissociation piston.

[0076] In some embodiments, when the tissue dissociator of Embodiment 1 above is used, the dissociation chamber provides a movement path for the dissociation piston, and the sliding sleeve limits its maximum stroke along the movement path.

[0077] In other Embodiments, when the tissue dissociator of Embodiment 2 above is used, the piston activity space 81 and the chamber wall of the dissociation chamber 8 together provide a movement path for the dissociation piston, and the top of the piston activity space 81 limits its maximum stroke along the movement path.

[0078] In other embodiments, when the tissue dissociator of Embodiment 3 above is used, it is only necessary to connect the base 3 and the lower sliding sleeve 102 in a sterile collection environment, and then connect the lower sliding sleeve 102 and the upper sliding sleeve 101, as well as the lower dissociation piston rod 202 and the upper dissociation piston rod 201.

[0079] When the base 3 and the sliding sleeve 1 are connected, the inlet pipe 4 and the outlet pipe 5 are in an initial state, that is, a sealed state, so that the formed internal dissociation chamber is a sealed sterile environment without risk of contamination.

[0080] S3. Using the tube connecting machine to connect the culture fluid bag and the sterile culture bag respectively to the inlet pipe 4 and the outlet pipe 5, so that the culture fluid bag and the sterile culture bag are connected with the dissociation chamber.

[0081] In some embodiments, this step S3 can be completed in a sterile collection environment. Of course, in order not to additionally occupy more space of the sterile collection environment, this step S3 can also not be completed in the sterile collection environment, but in any place outside the sterile collection environment. Accordingly, such any place can also be called the dissociation operating environment. Of course, those of ordinary skill in the art can understand that such any place does not include heavily contaminated places, such as next to garbage stations or garbage bins. After the culture fluid bag and the sterile culture bag are respectively connected to the inlet pipe 4 and the outlet pipe 5 using the tube connecting machine, the culture fluid bag is connected with the inlet pipe 4, and the sterile culture bag is connected with the outlet pipe 5. Therefore, the culture fluid bag, the dissociation chamber, and the sterile culture bag form a passage.

[0082] In some embodiments, the sterile culture bag pre-stores a first preset volume of sterile gas, so as to provide the possibility for the dissociation piston rod to move freely in the sealed environment in the subsequent process.

[0083] In some embodiments, before connecting the tubes with the tube connecting machine, clamps can also be used to clamp the tubes of the culture fluid bag and the sterile culture bag, or clamp the inlet pipe and the outlet pipe of the tissue dissociator.

[0084] S4. Squeezing the culture fluid bag to squeeze the second preset volume of culture fluid into the dissociation chamber, and then blocking the inlet passage between the culture fluid bag and the dissociation chamber.

[0085] In some embodiments, if the above two-chamber culture fluid bag is not used, the pressure volume can be controlled manually.

[0086] In other embodiments, when the two-chamber culture fluid bag is used, the second preset volume of culture fluid required in the actual dissociation process can be pre-stored in the first chamber of the culture fluid bag, thereby avoiding errors caused by manual operation (for example, if squeezed too much, the force of the piston during the dissociation process may be weakened, thereby extending the dissociation time, and even causing the culture fluid to overflow into the top space of the piston; if pressed too little, the buffering effect is small, which may affect the dissociation effect). Specifically, squeezing the first chamber in the direction of the outlet tube causes the second preset volume of culture fluid stored in the first chamber to enter the dissociation chamber through the outlet tube. As mentioned previously, since a partition film is arranged between the two chambers, it is also possible not to use clamps to block the inlet passage between the culture fluid bag and the dissociation chamber.

[0087] S5 Driving the dissociation piston rod 2 to dissociate the tissue to be dissociated.

During dissociation, the dissociation portion on the dissociation piston rod 2/dissociation piston 7 dissociates the tissue to be dissociated. During the push-pull process of the dissociation piston rod 2, the gas in the dissociation chamber is discharged into the sterile culture bag, avoiding the influence of air pressure in the dissociation chamber, so that the dissociation work can proceed smoothly.

[0088] Of course, preferably, a certain amount of sterile gas can also be pre-stored in the sterile culture bag, and during the dissociation process, the gas volume in the sterile culture bag is inversely correlated with the distance between the dissociation piston rod 2 and the bottom of the dissociation chamber. That is, when the dissociation piston rod 2 is pushed toward the dissociation chamber, gas enters the sterile culture bag, so that the gas volume in the sterile culture bag gradually increases. Correspondingly, when the dissociation piston rod 2 is pulled away from the dissociation chamber, gas enters the dissociation chamber and the sliding sleeve 1 from the sterile culture bag, that is, the gas volume in the sterile culture bag gradually decreases.

[0089] S6. After dissociation is completed, squeezing the culture fluid bag to cause the remaining culture fluid in the culture fluid bag to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag. After the dissociation work is completed, the culture fluid bag is squeezed manually or by other means, so that the culture fluid is sprayed into the dissociation chamber. The culture fluid has a certain pressure, which can flush the dissociation chamber and flush the tissue attached to the wall of the dissociation chamber along with the culture fluid into the sterile culture bag, thereby completing tissue dissociation and transfer in a sealed environment.

[0090] In some embodiments, squeezing the second chamber causes the partition film between the first chamber and the second chamber to open, so that the culture fluid in the second chamber enters the first chamber. Then, squeezing the first chamber repeatedly in the direction of the outlet tube causes the second preset volume of culture fluid to enter the dissociation chamber through the outlet tube, flushing the dissociation chamber and flushing the dissociated tissue into the sterile culture bag.

[0091] Of course, in other embodiments, in order to reduce the residue of tissue particles in the dissociation chamber, after squeezing the first chamber so that the culture fluid enters the dissociation chamber, the tissue dissociator can be oscillated or shaken, causing most of the tissue particles attached to the chamber wall of the dissociation chamber to detach from the chamber wall and gather at the bottom of the dissociation chamber. Then, by placing the tissue dissociator at an angle (for example, when the tissue dissociator is tilted, the angle between the centerline of the dissociation chamber and a horizontal line is 0° - 45° ; of course, specifically, it can be tilted toward the outlet pipe side, or toward the inlet pipe side, so as to flush the two side walls and the bottom of the dissociation chamber), and squeezing the first chamber repeatedly, the culture fluid under the action of squeezing and gravity flushes the bottom of the dissociation chamber, flushing the dissociated tissue particles into the sterile culture bag.

[0092] S7. Blocking the outlet passage between the sterile culture bag and the dissociation chamber, and separating the sterile culture bag from the tissue dissociator, such that the sterile culture bag after separation is in a sealed state. In some embodiments, a heat sealer can be directly used to block the outlet passage, so that the sterile culture bag is automatically separated from the tissue dissociator while sealing the inlet tube, so that the sterile culture bag is in a sealed state, thereby enabling direct sterile culture in the sterile culture bag without further transfer.

[0093] It should be noted that, in this text, the terms “comprise,” “include,” or any other variation thereof are intended to cover non-exclusive inclusion, so that a process, method, article, or device including a series of elements not only includes those elements, but also includes other elements not explicitly listed, or further includes

inherent elements of such a process, method, article, or device. Without further limitation, an element defined by the phrase “comprising one ...” does not exclude the existence of additional identical elements in the process, method, article, or device including the element.

[0094] The above embodiments are described with reference to the figures, but the present application is not limited to the specific embodiments described above. The specific embodiments described above are merely illustrative, not restrictive. Those of ordinary skill in the art, in light of the present application, can make many forms without departing from the spirit of the present application and the scope protected by the claims, and these all fall within the protection of the present application.

CLAIMS

Claim 1. A tissue dissociation system, comprising:

a tissue dissociator, a culture fluid bag, a sterile culture bag, and a tube connecting machine used to separately connect an outlet tube of the culture fluid bag and an inlet tube of the sterile culture bag with the tissue dissociator; in an initial state, free ends of the outlet tube and the inlet tube are in a sealed state, wherein:

the tissue dissociator comprises: a sliding sleeve, a base detachably connected to one end of the sliding sleeve, and a dissociation piston rod axially movable to the base within an inner space of the sliding sleeve; when the base is connected with the sliding sleeve, the base and the axial inner space of the sliding sleeve form a dissociation chamber for providing an axial movement path for the dissociation piston rod, an end of the dissociation piston rod close to the base being provided with a dissociation portion for dissociating tissue; the dissociation chamber is connected to an inlet pipe and an outlet pipe, ends of the inlet pipe and outlet pipe not connected to the dissociation chamber being sealed;

an end of the dissociation piston rod is arranged with a dissociation piston, and the dissociation portion is mounted at an end of the dissociation piston; the diameter of the dissociation piston is larger than the diameter of the dissociation piston rod;

the sliding sleeve comprises detachable upper and lower sections, and the dissociation piston rod comprises detachable upper and lower sections; the lower dissociation piston rod is sleeved with an elastic sealing sleeve;

the sealing sleeve is in the shape of an inverted bowl, with the large end connected inside the sliding sleeve, and the small end connected on the lower dissociation piston rod, so that a connection portion between the dissociation piston rod and the sliding sleeve is sealed.

Claim 2. The tissue dissociation system according to Claim 1, wherein the inlet pipe and the outlet pipe are symmetrically arranged on two sides of the dissociation chamber, and the inlet pipe and the outlet pipe are arranged slanted, so that an extension line of a respective centerline of each of the inlet pipe and the outlet pipe passes through the bottom of the dissociation chamber.

Claim 3. The tissue dissociation system according to Claim 1, wherein a return spring is sleeved on the dissociation piston rod; one end of the return spring abuts against the dissociation piston rod, and the other end abuts against a spring seat in the sliding sleeve.

Claim 4. The tissue dissociation system according to Claim 1, wherein an annular sealing ring is arranged on the dissociation piston rod; and/or, the upper end of the dissociation piston rod extends out of the sliding sleeve and is arranged with a pressing disc configured for operation.

Claim 5. The tissue dissociation system according to Claim 1, wherein the culture fluid bag comprises a first chamber and a second chamber; the first chamber is connected to an end of the outlet tube; a partition film is arranged between the first chamber and the second chamber; the partition film can be opened under external force, so that the first chamber and the second chamber are connected; wherein, the first chamber pre-stores a second preset volume of culture fluid less than a third preset volume of culture fluid pre-stored in the second chamber.

Claim 6. A tissue dissociation method, applicable to the tissue dissociation system of any one of Claims 1-5, wherein:

separating the base and the sliding sleeve, which were pre-placed in a sterile collection environment, and placing obtained tissue to be dissociated into the base, and then connecting the base and the sliding sleeve, so that a dissociation chamber is formed between the base and the axial inner space of the sliding sleeve for providing an axial movement path for the dissociation piston;

using the tube connecting machine to connect the culture fluid bag and the sterile culture bag respectively to the inlet pipe and the outlet pipe of the dissociation chamber, so that the culture fluid bag and the sterile culture bag are connected with the dissociation chamber;

squeezing the culture fluid bag, causing the second preset volume of culture fluid stored in the culture fluid bag to enter the dissociation chamber through the inlet pipe;

driving the dissociation piston rod to dissociate the tissue to be dissociated, such that during the entire dissociation process, an outlet path between the sterile culture bag and the dissociation chamber remains unobstructed;

after dissociation is completed, squeezing the culture fluid bag to cause the remaining culture fluid in the culture fluid bag to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag;

blocking the outlet passage between the sterile culture bag and the dissociation chamber, and separating the sterile culture bag from the tissue dissociator, such that the inlet tube of the sterile culture bag after separation is in a sealed state.

Claim 7. The tissue dissociation method according to Claim 6, wherein, the step of squeezing the culture fluid bag to cause the second preset volume of culture fluid to enter the dissociation chamber specifically comprises the following steps:

squeezing the first chamber of the culture fluid bag in the direction of the outlet tube on the culture fluid bag, causing the second preset volume of culture fluid stored in the first chamber to enter the dissociation chamber through the outlet tube; and/or,

after dissociation is completed, the steps of squeezing the culture fluid bag to cause the remaining culture fluid in the culture fluid bag to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag comprises the following steps:

squeezing the second chamber of the culture fluid bag, causing the partition film between the first chamber and the second chamber to open, thereby causing the third preset volume of culture fluid prestored in the second chamber to enter the first chamber; repeatedly squeezing the first chamber in the direction of the outlet tube, causing the third preset volume of culture fluid to enter the dissociation chamber through the outlet tube to flush the dissociation chamber and flush the dissociated tissue into the sterile culture bag.

Claim 8. The tissue dissociation method according to Claim 7, wherein, the steps of repeatedly squeezing the first chamber along the direction of the outlet tube specifically comprises:

squeezing the first chamber to cause the culture fluid to enter the dissociation chamber; oscillating or shaking the tissue dissociator, causing most of the tissue particles attached to the chamber wall of the dissociation chamber to detach from the chamber wall and gather at the bottom of the dissociation chamber;

placing the tissue dissociator at an angle and squeezing the first chamber repeatedly, causing the culture fluid under the action of squeezing and gravity to flush the bottom of the dissociation chamber, thereby flushing the dissociated tissue particles into the sterile culture bag.