SYSTEMS AND METHODS FOR PROCESSING CELLS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to and the benefit of, and incorporates herein by reference in its entirety, U.S. Provisional Patent Application No. 61/557,127, which was filed on November 8, 2011.

TECHNICAL FIELD

[0002] In various embodiments, the present invention relates to systems and methods for processing tissue to isolate and collect target cells.

BACKGROUND OF THE INVENTION

[0003] Purification of viable cells from a tissue sample can be a laborious process that involves dissection and other manual manipulation and processing steps, as well as, in some cases, cell culturing. Maintaining sterility of the cells during the purification process is also an important concern. Although laminar hoods can be used to maintain sterility, they suffer a number of disadvantages. For example, such hoods are expensive, relatively immobile, cumbersome to work with, and consume valuable laboratory space. Efficiency of the cell purification process is another concern that further complicates the purification process. Isolating rare cells, such as stem cells, from a tissue sample requires an efficient process to recover as many of the cells as possible.

[0004] There remains a need for a practical, cost-effective, sterile, and efficient mechanism and method for extracting and collecting cells, such as stem cells, for advancing potential therapies that rely on the administration of these cells.

SUMMARY ~~OF THE INVENTION~~

[0005] The present ~~invention~~ disclosure efficiently and cost-effectively extracts and collects cells from a tissue. The inventors have discovered that the tissue can be effectively fragmented and the resulting cells can be purified using a system or kit with multiple components. An advantage of the present ~~invention~~ disclosure is that tissue processing takes place in a closed system such that

sterility can be maintained throughout the process, even if certain components are removed during processing, for example through the use of valves, clamps, and heat seals. Furthermore, any or all of the steps can be automated or manually accomplished, according to the specific needs of the application or the user.

[0006] Thus, in one aspect, the ~~invention~~ present disclosure relates to a tissue mincing tool. The tissue mincing tool includes a compartment for a tissue sample, a cutting surface at one end of the compartment, and a sterile, sealed container. The cutting surface separates the compartment from the sterile, sealed container, such that a tissue sample that passes through the cutting surface can be deposited within the container. The cutting surface can be dimensioned to mince the tissue sample into fragments having an average cross-section no greater than four square millimeters. For example, the number of square millimeters may be no greater than 3, 2, 1, 0.5, 0.3, 0.2, 0.1, or 0.05 in various embodiments of the present disclosure~~invention~~. The tissue mincing tool can also include a second cutting surface for further reducing the average cross-section of the fragments. The cutting surface of the tissue mincing tool can include an automated cutting system. For example, the cutting surface may include semi-automatic scissors. One or more mincer screens may be positioned in proximity to the cutting surface. The tissue mincing tool may further include a suction cup for stabilizing the tool during operation thereof. The tissue mincing tool also or alternatively can include a fluid conduit in communication with the sterile, sealed container, and a separator unit such as one or more filters within the fluid conduit. The sterile, sealed container optionally includes at least one sealed access port permitting the sterile introduction of a fluid into the container.

[0007] In the tissue mincing tool, the compartment for the tissue sample can incorporate one or more features to facilitate the application of a force to the tissue sample, impelling it beyond the cutting surface and into the sterile, sealed container. For example, a portion of the compartment can be shaped to receive a solid member to press the tissue sample. Such a solid member can optionally be included with the tissue mincing tool, whether connected to it or provided as a separate component.

[0008] In one embodiment, a portion of the compartment near the cutting surface has a substantially constant cross-section, such that a comparably shaped solid member may be introduced into the compartment and fill that portion, while pressing the tissue sample into or

through the cutting surface. In another embodiment, the compartment near the cutting surface has a tapered or cone-shaped end. In some embodiments, an interior surface of the compartment is threaded, such that a threaded solid member may be guided into the compartment. In some embodiments, the compartment also includes a gasket, which can provide an improved seal when a solid member is introduced into the compartment. In yet another embodiment, the tissue mincing tool includes a shaft crank for moving the cutting surface towards the tissue sample.

[0009] In an aspect, the present disclosure provides a kit comprising: a tissue mincing tool as disclosed herein; and a solid member shaped to mate with and to fill the portion of the compartment, the solid member being movable within the portion of the compartment to impel the tissue sample therein through the cutting surface. The ~~invention~~ present disclosure also provides methods of using any of the above-described tissue mincing tools by impelling the tissue sample through the cutting surface of the tool. The present disclosure ~~invention~~ provides methods of mincing a tissue sample and optionally injecting an enzyme into the sterile, sealed container, such that the enzyme enhances the digestion of the minced tissue. In an aspect, the present disclosure provides a method of mincing a tissue sample, the method comprising: impelling the tissue sample through the cutting surface of a tissue mincing tool as disclosed herein. In another aspect, the present disclosure provides a method of digesting a tissue sample, the method comprising: mincing the tissue sample as disclosed herein; and injecting an enzyme into the sterile, sealed container, whereby the enzyme digests the minced tissue. In yet another aspect, the present disclosure provides a method of separating cells from a tissue sample, the method comprising: digesting the tissue sample as disclosed herein, and removing undigested tissue. The enzyme can be a protease, such as collagenase, hyaluronidase, or dispase, separately or in combination. These steps can optionally be incorporated into a method of separating cells from the tissue sample by mincing and/or digesting the tissue sample and removing fragments larger than about 40 microns (*e.g.* fragments retained by a filter having a pore size of about 500 microns, or fragments retained by a filter having a pore size of about 300 microns, or fragments retained by a filter having a pore size of about 250 microns, or fragments retained by a filter having a pore size of about 150 microns, or fragments retained by a filter having a pore size of about 100 microns, or fragments retained by a filter having a pore size of about 70 microns, or fragments retained by a filter having a pore size of about 40 microns). These larger fragments, referred to herein as "undigested tissue," can be removed by filtering or sedimentation. These methods are effective for purifying cells from any of a variety of

solid tissues. For example, the methods described herein can separate cells, such as stem cells, from fat tissue or afterbirth tissue, such as placental or umbilical cord tissue or, more specifically, a tissue comprising Wharton's Jelly. In some embodiments, the tissue sample is substantially free of blood vessels, which can optionally be dissected from a tissue before the tissue is placed in the compartment.

[0010] In another aspect, the ~~invention~~ present disclosure relates to a cell collection method including sedimenting cells in a cell collection device. The cell collection device includes a sterile container for a fluid including cells and a fluid passageway in communication with the sterile container. The fluid passageway includes a cell capture zone, such that the volume of the cell capture zone can be less than 5% of the volume of the sterile container and the sterile container

and the cell capture zone can be configured such that sedimentation of the cells from the fluid in the sterile container concentrates the cells in the cell capture zone. This aspect or any of the following aspects can have any of the following embodiments. The cell collection device further includes a second sterile container in communication with the fluid passageway and/or a removable clamp to regulate passage of material into the second sterile container. The second sterile container can be heat-sealable and/or a bag. The cell collection method can also include centrifuging the cell collection device to accelerate sedimentation of the cells, and the cells can be Wharton's Jelly stem cells. The fluid includes mechanically minced and/or enzymatically digested umbilical cord tissue. The cell collection method can further include adding a cryoprotectant to the cells; the cryoprotectant includes DMSO, albumin, and/or dextran. The method can also include adding autologous plasma to the cells.

[0011] In still another aspect, the present disclosure ~~invention~~ relates to a cell collection device having a fluid including cells, a sterile container for housing the cells, and a fluid passageway in communication with the sterile container. The fluid passageway includes a cell capture zone, wherein the volume of the cell capture zone can be less than 5% of the volume of the sterile container and the sterile container and the cell capture zone can be configured such that sedimentation of the cells from the fluid in the sterile container concentrates the cells in the cell capture zone. This aspect or any of the following aspects can have any of the following embodiments. The cell collection device also or alternatively includes a second sterile container in communication with the fluid passageway and/or a removable clamp to regulate passage of material into the second sterile container. The second sterile container can be heat-sealable and/or a bag.

[0012] In yet another aspect, the present disclosure ~~invention~~ relates to a cell collection device having a sterile container for a fluid comprising cells, such that the sterile container can be adapted for use in sedimentation, and a fluid passageway in communication with the sterile container. The fluid passageway includes first and second valves defining a cell capture zone therebetween, such that the volume of the cell capture zone can be less than 5% of the volume of the sterile container and the sterile container and the cell capture zone can be configured such that sedimentation of the cells from the fluid in the sterile container concentrates the cells in the cell capture zone. This aspect or any of the following aspects can have any of the following embodiments. The sterile container can be adapted for use in centrifugation. The cell collection device also or alternatively includes a second sterile container in communication

with the second valve and/or a removable clamp to regulate passage of material into the second sterile container. The second sterile container can be heat-sealable and/or a bag.

[0013] In still yet another aspect, the present disclosure ~~invention~~ relates to a cell collection device having a sterile container for housing the cells, a fluid passageway in communication with the sterile container, and a second sterile container in communication with the fluid passageway. The fluid passageway includes a cell capture zone, such that the volume of the cell capture zone is less than 5% of the volume of the sterile container and the sterile container and the cell capture zone can be configured such that sedimentation of the cells from the fluid in the sterile container concentrates the cells in the cell capture zone. The second sterile container includes an element selected from the group consisting of a bag, a heat-sealable container, and a removable clamp.

[0014] In another aspect, the present disclosure ~~invention~~ relates to cell collection device that includes a compartment for receiving a tissue; a cutting surface dimensioned to mince the tissue sample into fragments having an average cross-section no greater than four square millimeters; a sterile, sealed container for holding a suspension of the minced tissue, the sterile container having a volume at least ten times greater than the volume of the compartment for receiving a tissue; a filter bag in fluid communication with the sterile, sealed container; and a sedimentation bag in fluid communication with the filter bag. The filter bag contains at least one filter having a pore size sufficiently small to retain particles larger than about 250 μm. The sedimentation bag includes a tapered portion to promote the concentration of sedimented cells.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Other features and advantages of the present invention, as well as the invention itself, can be more fully understood from the following description of the various embodiments, when read together with the accompanying drawings, in which:

[0016] FIG. 1 schematically depicts a tissue mincing tool, according to an embodiment of the invention;

[0017] FIG. 2 is a schematic perspective view of a solid member of a tissue mincing tool, according to an embodiment of the invention;

[0018] FIG. 3 is a schematic perspective view of the solid member of FIG. 2;