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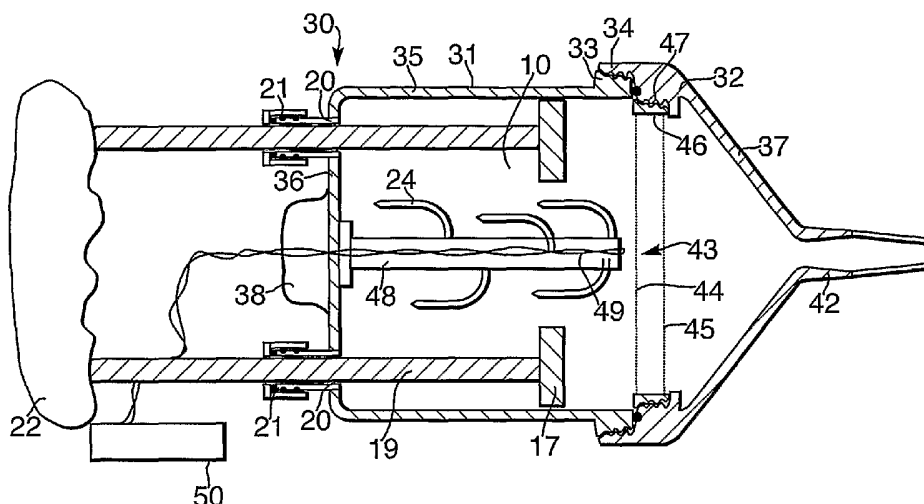
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(54) Title: **DIGESTION CHAMBER FOR CELL ISOLATION**



(57) **Abstract:** A digestion chamber (30) contains animal tissue during digestion of the animal tissue by an enzyme preparation to isolate cells from the animal tissue, most importantly the isolation of islets from human pancreatic tissue using collagenase. The digestion chamber has an annular piston (17) which is reciprocable and a plurality of hooks (24) for catching the tissue fixed to a mounting rod extending through the central opening in the annular piston. In use, reciprocation of the piston causes a reciprocating flow through the central opening. The tissue is repeatedly caught by the hooks, separated by the flow and released. This increases the effectiveness of the isolation of cells by separating fibrous tissue which is not digested by the enzyme preparation.

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Digestion Chamber For Cell Isolation

The present invention relates to the isolation of cells from animal tissue using a technique in which the tissue is digested by an enzyme preparation, typically collagenase. The animal tissue may be human tissue. Currently, the most important application of the present invention is to the isolation of islets (Islets of Langerhaus) from human pancreatic tissue.

Transplantation of such islets isolated from pancreatic tissue has proven to be an effective treatment for type 1 diabetes. To provide a source for transplantation, the conventional method for isolating islets relies on selective digestion of the pancreatic tissue using collagenase which is an enzyme delivered via the pancreatic duct. In particular, the collagenase digests and removes the peri-acinar fibrous tissue. Typically, to provide an automated and relatively-large scale isolation method, the pancreatic tissue is held in a digestion chamber and collagenase is continuously recirculated through the digestion chamber until a sufficient population of islets has been released.

However, collagenase has relatively little effect on the interlobular fibrous tissue within the pancreatic tissue. Therefore, in the conventional isolation method, the interlobular fibrous tissue is broken down by mechanical means. In particular, a number of balls, typically glass marbles or stainless steel balls, are placed within the digestion chamber together with the pancreatic tissue. During recirculation of the collagenase, the digestion chamber is shaken by connecting the digestion chamber to a mechanical shaker. The shaking is relatively vigorous, typically at around 5Hz. The shaking causes the balls to impact on the pancreatic tissue which assists in breaking down the interlobular fibrous tissue to release the islets.

It would be desirable to improve the effectiveness of the conventional automated method, either by increasing the yield in terms of the number of islets isolated, or by increasing the size of the clusters of islets released into the fluid, or both.

Accordingly to the present invention, there is provided a digestion chamber for containing animal tissue during digestion of the animal tissue by an enzyme

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preparation to isolate cells from the animal tissue, the digestion chamber comprising:

walls defining a cavity for containing animal tissue and a fluid containing said enzyme preparation;

tissue catching means disposed within the cavity for catching animal tissue

5 held in the cavity; and

fluid movement means capable of creating a reciprocating flow of fluid within the cavity relative to the tissue catching means for causing the tissue to be repeatedly caught and released by the tissue catching means and for separating the tissue caught by the tissue catching means.

10 A digestion chamber in accordance with the present invention may be used to contain animal tissue during digestion by an enzyme preparation to isolate cells from the animal tissue. The digestion chamber in accordance with the present invention provides for more effective isolation of the cells, as compared to the use of a conventional digestion chamber containing balls and being shaken, as described
15 above. It has been appreciated that with such a conventional digestion chamber, the shaken balls are not ideal for breaking down the interlobular fibrous tissue. In particular, the balls are not particularly effective at separating tissue. Furthermore, it is believed that the impact of the balls on the tissue can actually damage the islets as the interlobular fibrous tissue is broken down. This may reduce the size of the
20 clusters of islets released into the fluid. Furthermore, the impacts may actually cause damage to the islets which prevent them being as effective for the treatment of diabetes after transplantation.

The digestion chamber in accordance with the present invention avoids these disadvantages of the conventional digestion chamber by providing for a different
25 mechanical process for separating the interlobular fibrous tissue. In particular, the chamber is provided with tissue catching means for catching the tissue and fluid movement means for causing movement of the fluid relative to the tissue catching means. In use, tissue is caught on the tissue catching means and the relative movement of the fluid separates the caught tissue. In particular, the relative
30 movement of the fluid applies shear forces to the fibrous strands of the caught tissue,

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which shear forces separate the tissue. The effect of the digestion chamber in use is thought to be similar to the effect of manually teasing the tissue apart with forceps. This mechanical separation process is effective in separating the tissue.

The reciprocating flow is advantageous because it causes a process in which

5 (a) the flow in the a first direction causes the tissue to become caught by the tissue catching means and separated by the flow and (b) the flow in the second direction causes the tissue to be released. This process can occur repeatedly. Such repeated catching, separation and release of the tissue is particularly effective because the tissue tends to move randomly and be caught in different locations and orientations,

10 whereby it is subject to repeatedly changing separation forces.

The digestion chamber in accordance with the present invention is capable of increasing the effectiveness of the isolation of cells from the animal tissue as compared to the conventional digestion chamber as follows. Higher yields are possible because the amount of damage to the islets is reduced as compared to the

15 damage produced by impacting the tissue by shaken balls. In addition, it is possible to obtain larger clusters of islets than in the conventional method which involves the tissue being impacted by shaken balls.

Preferably, the tissue catching means comprises a plurality of hooks.

Hooks are particularly effective for catching the tissue. However, in

20 principal, the tissue catching means could be any structure which catches the tissue, for example simple pieces of straight wire, a mesh, a serrated edge, or grooves, although this list is not exhaustive.

Preferably, the hooks each comprise a bent wire.

Hooks in the form of a bent wire are simple and easy to manufacture. On the

25 other hand, any other form of hook could be used.

Preferably, the tissue catching means is fixed within the digestion chamber and the fluid movement means comprises fluid flow means for causing flow of the fluid within the cavity.

Having such a fixed tissue catching means and a fluid flow means is

30 advantageous because it is effective in separating the tissue. In addition, the flow of

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fluid is useful in the case that the digestion chamber has an inlet and an outlet covered by a filter to contain the tissue in the chamber, because in that case the flow created by the fluid flow means can assist in clearing the filters and preventing blockage by pieces of the tissue.

- 5 Advantageously, the digestion chamber has an inlet and an outlet at opposite ends of the digestion chamber along the direction of the reciprocating flow.

By this arrangement, the overall flow through the digestion chamber from the inlet to the outlet is in the same direction as the reciprocating flow. The overall flow between the inlet and the outlet is generally slow, for example being the circulation
10 flow in the conventional method, and is in particular slow as compared to the reciprocation flow which separates the tissue. Arranging for the overall flow to be in the same direction as the reciprocation flow generated by the fluid flow means increases the effectiveness of the separation of the tissue and hence the isolation of the cells.

- 15 Preferably, the fluid flow means comprises a piston which is reciprocable within the cavity, the piston extending partially across the cavity so that movement of the piston causes fluid displaced by the piston to flow past the piston.

A piston is simple, yet is capable of producing large flows within the cavity. In particular, as the piston is moved, fluid is displaced from one side of the piston to
20 the other side of the piston, which causes the displaced fluid to flow past the side of the piston. As the gap is smaller than the cross-section of the cavity, the fluid is caused to flow through the gap relative to the tissue catching means. As the area of the gap decreases relative to the area swept out by the piston, the speed of the flow relative to the tissue catching means increases. Also, in forcing the fluid through the
25 gap, the piston mixes the fluid and the animal tissue entrained therein. This assists in causing the tissue to be caught in different locations and orientations during the repeated catching and release, which in turn assists the separation process.

- To make best use of this flow, the tissue catching means may be disposed beside the piston along the reciprocation path of the piston, preferable along
30 substantially the entire reciprocation path.

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Advantageously, the piston is annular with a central opening and the tissue catching means is disposed in the central opening of the piston.

Such an arrangement is advantageous, because it maximises the amount of fluid flowing for a given displacement of the piston. This is because none of the
5 fluid flows adjacent a static wall of the chamber which would apply a shear force to the flowing fluid and slow the flow.

The tissue catching means may be fixed to a mounting rod extending through the central opening of the piston. This is a particularly convenient arrangement for the tissue catching means.

10 The central opening of the piston may be circular although any other shape is possible.

To provide for manual operation of the fluid flow means, the fluid flow means may further comprise a handle for driving reciprocation of the piston, the handle being disposed externally to the chamber and connected to the piston by at
15 least one connecting rod extending through the walls of the chamber. However, such a handle is not essential. The piston may alternatively be driven mechanically, for example by an electric motor.

Although the reciprocating flow advantageously improves the separation process, it is still possible to achieve a degree of separation without it. Therefore
20 according to a further aspect of the present invention, there is provided a digestion chamber for containing animal tissue during digestion of the animal tissue by an enzyme preparation to isolate cells from the animal tissue, the digestion chamber comprising:

walls defining a cavity for containing animal tissue and a fluid containing
25 said enzyme preparation;

tissue catching means disposed within the cavity for catching animal tissue held in the cavity; and

fluid movement means capable of causing movement of the fluid relative to the tissue catching means for separating the tissue caught by the tissue catching
30 means.

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To allow better understanding, embodiments of the present invention will now be described by way of non-limitative example with reference to the accompanying drawings, in which:

Fig. 1 is an exploded, cross-sectional side view of a first digestion chamber;

5 Fig. 2 is an end view of the first digestion chamber taken from the left-hand side in Fig. 1.

Fig. 3 is an end view of the first digestion chamber taken from the right-hand side in Fig. 1;

Fig. 4 is a cross-sectional side view of a second digestion chamber;

10 Fig. 5 is an end view of the second digestion chamber taken from the left-hand side in Fig. 5 with the handle of the second digestion chamber omitted; and

Fig. 6 is a partial cross-sectional view of the inlet of the second digestion chamber taken along the line VI-VI in Fig. 5.

A first digestion chamber 1 is illustrated in Fig. 1 which is an exploded,
15 cross-sectional view taken axially along the length of the digestion chamber 1.

The digestion chamber 1 comprises a cylinder 2 and first and second end-plates 3 and 4 which close the open ends of the cylinder 2. The cylinder 2 is made of glass and the first and second end-plates 3 and 4 are made of Teflon. Annular seals 5 are disposed between the ends of cylinder 2 and each of the first and second end
20 plates 3 and 4.

A threaded tie-rod 6 extends centrally along the axis of the cylinder 2 and protrudes through holes 7 and 8 formed centrally in the end plates 3 and 4, respectively. Externally knurled nuts 9 are screwed onto each end of the tie-rod 6 to secure the first and second end plates 3 and 4 onto the open ends of the cylinder 2.

25 Thus, the cylinder 2 itself and first and second end plates 3 and 4 together constitute walls of the digestion chamber 1 which define an internal cavity 10. In use, the internal cavity 10 contains animal tissue from which cells are to be isolated and a physiologically compatible fluid containing collagenase.

The first end plate 3 has a pair of inlet openings 11 arranged eccentrically
30 from the central opening 7 to constitute an inlet to the cavity 10. Similarly, the

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second end plate 4 has an outlet opening 12 arranged eccentrically from the central opening 8 to constitute an outlet for the cavity 10. In use, a fluid enzyme preparation such as collagenase is caused to flow through the digestion chamber 1 to digest the animal tissue held in the cavity 10, the enzyme preparation flowing into the cavity
5 through the inlet openings 11 and out of the cavity 10 through the outlet opening 12.

Inside the cavity 10, the digestion chamber 1 is provided with a first filter 13 arranged adjacent the first end plate 3 and a second filter 14 arranged adjacently second end plate 4. The first and second filters 13 and 14 are both circular and extend across the entire cavity 10. The first filter 13 therefore covers the inlet
10 openings 11 and the second filter 14 covers the outlet opening 12, to contain animal tissue within the cavity 10. The first inlet 13 is a mesh sheet. The second outlet 14 comprises an inner mesh sheet 15 having the same pore size of the mesh sheet of the first filter 13, in series with an outer mesh sheet 16 having a smaller pore size than the first mesh sheet 15.

15 For creating a flow of fluid within the cavity 10, the digestion chamber 1 has a piston 17. The piston 17 is annular with a central opening 18, so extends only partially across the cavity 10. The central opening 18 is circular. The outer edge of the piston 17 is circular so that it sits flush against the internal surface of the cylinder 2 which forms a wall of the cavity 10. The piston 17 is free to reciprocate along the
20 length of the cylinder 2 between the first and second filters 13 and 14. Thus, the gap between the first and second filters 13 and 14 may be considered as a reciprocation path for the piston 17. Movement of the piston 17 displaces fluid in the volume of the cavity 10 swept out by the piston 17 from one side of the piston 17 to the other. The displaced fluid is therefore cause to flow through the central opening 18 of the piston
25 17. As the opening 18 is surrounded by the piston 17, none of the fluid flowing past the piston 17 flows adjacent to an internal surface of the cylinder 2 which would otherwise impart a shearing force on the fluid and slow the flow. As the piston 17 fits flush against the cylinder 2 along its reciprocation path, all the fluid displaced by the piston 17 flows through the central opening 18 without any fluid escaping around
30 the outer edge of the piston 17.

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The piston 17 is connected to a handle 22 disposed externally of the cavity 10 by two connecting rods 19 which are attached at one end to the piston 17 and at the other end to the handle 22. The connecting rods 19 extend from the piston 17 parallel to the axis of the cylinder 2 out of the cavity 10 through ports 20 for in the first end plate 3. The ports 20 are each provided with a seal 21 to prevent escape of fluid between the connecting rods 19 and their respective ports 20. Using the handle 22, it is possible to manually drive reciprocation of the piston 17.

The tie-rod 6 supports a mounting post 23 which is attached by an internal screw thread onto the tie-rod 6. The mounting post 23 has a plurality of hooks 24 fixed thereto. The hooks 24 are disposed within the central opening 18 of the piston 17. All the hooks 24 are within the volume swept out by the central opening 18 during reciprocation of the piston 17 so that the hooks 24 do not obstruct the piston 17. The hooks 24 are spread along substantially the entire reciprocation path of the piston 17. The hooks 24 are also spread around the mounting post 23.

Each hook 24 comprises a piece of wire bent into a shape having a bight portion 25 attached to the mounting post 23 and a straight portion 26 extending from the free end 27 of the hook 24 parallel to the direction of reciprocation of the piston 17. The free ends 27 of the hooks 24 are pointed to assist the catching of tissue.

On reciprocation of the piston 17, the fluid flowing through the central opening 18 flows past the hooks 24. Such flow of the fluid relative to the hooks 24 occurs because the area of the aperture is less than the cross-section of the cavity 10. The speed of flow of the fluid relative to the hooks 24 increases as the ratio of the area of the opening 18 to the area of the piston 10 decreases, so it is possible to achieve high flow speeds, greater than the speed of the piston 10 when the ratio is less than one. When the fluid flows through the central opening 18 in a first direction towards the free ends 27 of the hooks 24, the pieces of tissue entrained in the fluid become caught by the hooks 24 and are retained on the bight portions 25 of the hooks 24. Subsequent flow of the fluid imparts a shear force to the fibrous strands of the tissue caught by the hooks 24. This is effective to separate the tissue. The effect is thought to be similar to the effect of manually teasing the tissue apart with forceps.

Subsequently, when the fluid flows through the central opening 18 in the opposite direction away from the free ends 27 of the hooks 24, this causes the caught tissue to be released from the hooks 24.

Repeated reciprocation of the piston 17 causes the separation process to be repeated. Each time, the random movement of the pieces of tissue causes them to be caught in different locations and orientations which increases the effectiveness of the separation. This random movement is increased by the fact that the fluid is forced through the opening 18 in the piston, thereby causing turbulence 17. As the opening 18 is narrower than the cavity 10 the flow is turbulent and reorients the entrained tissue to a greater extent than if the reciprocating flow was laminar.

In addition, the flow generated by the piston 17 has the advantage of dislodging tissue from the first and second filters 13 and 14 which prevents them becoming clogged.

In the digestion chamber 1, the hooks 24 are all aligned in the same direction, in particular being directed towards the end of the cavity 10 having the inlet openings 11. This has the advantage that the overall flow through the digestion chamber 1 from the inlet openings 11 to the opening 12 assists in catching of the tissue on the hooks 24. However, in principal, the hooks could all be aligned in the opposite direction or the hooks 24 could be aligned in different directions.

In general, the form and shape and alignment of the hooks 24 could be changed to achieve a similar effect, but this particular form for the hooks 24 has a number of advantages. Formation from a bent wire makes the hooks 24 simple to manufacture. The alignment of the straight portion 26 parallel to the direction of reciprocation of the piston 17 assists in catching of tissue on a hook 24, because the straight portion 26 is aligned with the direction in which fluid flows through the central opening 18. The simple form of the hooks 24 as a piece of wire, in particular without any barbs, also provides the advantage of assisting the release of material by flow of the fluid in the cavity 10 in the opposite direction.

A second digestion chamber 30 is illustrated in Fig. 4 which is a cross-sectional view taken axially along the length of the second digestion chamber 30.

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The second digestion chamber 30 is similar in overall shape and operation to the first digestion chamber 1. Therefore, for brevity, in respect of elements of the second digestion chamber 30 which are the same as elements of the first digestion chamber 1, the same reference numerals will be used and a description thereof will not be repeated.

The second digestion chamber 30 is intended to be disposable after a single use. Accordingly, the body of the second digestion chamber 30 has a different form from that of the first digestion chamber 1. In particular, the second digestion chamber 30 comprises a first and second body portion 31 and 32, each having a respective threaded portion 33 and 34 by which the body portions 31 and 32 may be screwed together. Each of the first and second body portions 31 and 32 is integrally formed. The first body portion 31 comprises a cylindrical side wall 35 and a circular end wall 36 closing one end of the cylindrical side wall 35. The second body portion 32 has a conical wall 37 which closes the end of the cylindrical side wall 35 of the first body portion 31 opposite from the end wall 36. Accordingly, the side wall 35 and the end wall 36 of the first body portion 31 and the conical wall 37 of the second body portion 32 together constitutes walls defining the internal cavity 10.

As best seen in Figs. 5 and 6, the end wall 36 of the first body portion 31 has a protruding cylindrical recess 38 arranged eccentrically from the axis of the cylindrical side wall 35 and having an outlet 39 formed therein. The outlet 39 is formed as a flexible conduit to allow easy connection to a tube. A filter 40 is attached in the recess 38 by being screwed into a thread 41 formed on the internal cylindrical wall of the recess 38. The filter 40 is circular and covers the inlet 39.

The second body portion 32 is provided with an outlet 42 arranged centrally. The outlet 42 is formed as a flexible conduit to allow easy connection to a tube.

The second body portion 32 has a filter 43 formed by a pair of mesh screens 44 and 45 fitted in an annular screw ring 46 which is itself attached to the second body portion 32 by a thread 47 formed internally in the second body portion 32 at the wide end of the conical wall 37. The filter 43 is circular and covers the outlet 42.

Accordingly, the filters 40 and 43 of the second digestion chamber 30

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correspond to the filters 13 and 14 of the first digestion chamber 1.

Within the second digestion chamber 30, there is a piston 17 having the same form as the piston 17 of the first digestion chamber 1. In particular, the piston 17 is connected to a handle 22 by connecting rods 19 having the same arrangement as in
5 the first digestion chamber of Fig. 1, with the ports 20 and seals 21 through which the connecting rods 19 extend being formed in the end wall 36 of the first body portion 31.

The second digestion chamber 30 is provided with hooks 24 which are arranged in the same location and have the same form and alignment as the hooks 24
10 of the first digestion chamber 1. However, in the second digestion chamber 30, the hooks 24 are mounted on a mounting post 48 which is fixed to the centre of the end wall 36 of the first body portion 31. The mounting post 48 extends into the cavity 10 along the axis of the cylindrical wall 35 of the first body portion 31 parallel to the direction of reciprocation of the piston 17.

15 The second digestion chamber 30 is also provided with a thermocouple 49 mounted along the mounting post 48 and connected to an external electrical circuit 50 for monitoring the temperature of fluid within the cavity 10 of the digestion chamber 30. Such a temperature sensing arrangement is conventional within a known digestion chamber and may equally be applied to the first digestion chamber
20 1.

The operation of the second digestion chamber 30 is identical to the operation of the first digestion chamber 1, so a description thereof will not be repeated.

The first and second digestion chambers 1 and 30 are suitable for use in the conventional method of isolating islets from pancreatic tissue. In such a conventional
25 method, a physiologically compatible fluid having collagenase dissolved therein is used to digest the tissue to release the islets. The tissue is arranged in the digestion chamber. Collagenase is continuously recirculated through the digestion chamber, the collagenase being heated to a suitable temperature for digestion before supply to the digestion chamber. When a sufficient population of islets has been released, the fluid
30 containing the released islets is directed into a collection vessel. After that, the islets

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are usually subjected to further purification steps although such purification is not in itself relevant to the digestion chambers 1 and 30 of the present invention.

Such a conventional method is well known in the art and is, for example, described in US-5,079,160 and in Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp
5 DW : Automated method for isolation of human pancreatic islets . Diabetes 37: 413-420, 1988, which are both incorporated herein by reference.

Either one of the digestion chambers 1 and 30 is used in place of the conventional digestion chamber. The method is then performed in the conventional manner except that, as the fluid is recirculated through the digestion chamber 1 or 30,
10 the piston 17 is reciprocated. This has the effect of separating the tissue as described in detail above. Such separation assists the digestion by the collagenase and the release of the islets.

It is expected that the use of the digestion chamber in accordance with the present invention will increase the effectiveness of the isolation of cells from the
15 animal tissue, as compared to the use of a conventional digestion chamber. In particular, it is expected that high yields will be possible, because there will be less damage to the cells as compared to the damage by impacting the tissue by shaken balls. In addition, it is expected that it will be possible to obtain larger clusters of islets as compared to the conventional method in which tissue is impacted by shaken
20 balls.

Preliminary tests have been performed using the first digestion chamber 1 to isolate islets from pancreatic tissue. Experience processing five human pancreases has now demonstrated that in three out of the five pancreases, the first digestion chamber 1 produced a high yield of more than 200,000 islets which were remarkably
25 well separated and intact. The less effective results in two of the five pancreases is not unexpected because of the well known variability of human islet isolation, similar variability being very common within the conventional method. Therefore, the first digestion chamber 1 has also been used to isolate islets from porcine pancreatic tissue, for which donor factors can more easily be controlled. From these
30 tests, it has been confirmed that the new chamber can isolate porcine islets in large

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numbers, of more than 500 islets per gram of pancreas.

At present, this type of technique is very important for the isolation of islets from human pancreatic tissue because of the effectiveness of islets in treating diabetes by transplantation. In the future, it may become important for the isolation
5 of islets from tissue of non-human animals such as pigs for transplantation in humans. However, in principle, the digestion chamber could be used in the isolation of any type of cell from any type of animal tissue by any type of method using an enzyme preparation for digestion of the tissue.

Collagenase is preferred as an enzyme preparation and is conventionally used
10 for isolation of islets from pancreatic tissue. However, in principle collagenase could be replaced by any enzyme preparation effective to digest the tissue in question, or indeed by any agent effective to break down the tissue in question.

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Claims

1. A digestion chamber for containing animal tissue during digestion of the animal tissue by an enzyme preparation to isolate cells from the animal tissue, the
5 digestion chamber comprising:
walls defining a cavity for containing animal tissue and a fluid containing said enzyme preparation;
tissue catching means disposed within the cavity for catching animal tissue held in the cavity; and
10 fluid movement means capable of creating a reciprocating flow of fluid within the cavity relative to the tissue catching means for causing the tissue to be repeatedly caught and released by the tissue catching means and for separating the tissue caught by the tissue catching means.
- 15 2. A digestion chamber according to claim 1, wherein the tissue catching means comprises a plurality of hooks.
3. A digestion chamber according to claim 2, wherein the hooks each comprise a bent wire.
20
4. A digestion chamber according to claim 2 or 3, wherein the hooks are all aligned parallel to the direction of the reciprocating flow.
5. A digestion chamber according to any one of claims 2 to 4, wherein the hooks
25 are all aligned in same direction.
6. A digestion chamber according to any one of claims 2 to 5, wherein the cavity has an inlet and an outlet and the free ends of the hooks are directed towards the inlet.
30

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7. A digestion chamber according to any one of claims 2 to 6, wherein the hooks have a straight portion extending from their free ends parallel to the direction of the reciprocating flow.
- 5 8. A digestion chamber according to any one of claims 2 to 7, wherein the free ends of the hooks are pointed.
9. A digestion chamber according to any one of the preceding claims, wherein the tissue catching means is fixed within the digestion chamber and the fluid
10 movement means comprises fluid flow means for causing flow of the fluid relative to the cavity.
10. A digestion chamber according to any one of the preceding claims, wherein the digestion chamber has an inlet and an outlet at opposite ends of the digestion
15 chamber along the direction of the reciprocating flow.
11. A digestion chamber according to claims 9 or 10, wherein the fluid flow means comprises a piston which is reciprocable within the cavity, the piston extending partially across the cavity so that movement of the piston causes fluid
20 displaced by the piston to flow past the piston.
12. A digestion chamber according to claim 10, wherein the tissue catching means is disposed beside the piston along the reciprocation path of the piston.
- 25 13. A digestion chamber according to claims 11 or 12, wherein the piston is annular with a central opening and the tissue catching means is disposed in the central opening of the piston.
14. A digestion chamber according to claim 13, wherein the tissue catching
30 means is fixed to a mounting post extending through the central opening of the

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piston.

15. A digestion chamber according to claims 13 or 14, wherein the central opening of the piston is circular.

5

16. A digestion chamber according to any one of claims 13 to 15, wherein the walls of the cavity are flush to the outer edge of the piston along the reciprocation path of the piston.

10 17. A digestion chamber according to any one of claims 11 to 16, wherein the fluid flow means further comprises a handle for driving reciprocation of the piston, the handle being disposed externally to the chamber and connected to the piston by at least one connecting rod extending through the walls of the chamber.

15 18. A digestion chamber according to any one of the preceding claims, wherein the digestion chamber has an inlet and an outlet each covered by a filter to contain the tissue in the chamber.

19. A method of isolating cells from animal tissue comprising
20 containing the animal tissue in a digestion chamber according to any one of the preceding claims,

introducing into the digestion chamber a fluid having dissolved therein an enzyme preparation effective to selectively digest the animal tissue to release said cells, and

25 operating the fluid movement means to cause the animal tissue to be repeatedly caught and released by the tissue catching means and separated by the flow of the fluid relative to the tissue catching means.

20. A method according to claim 19, wherein said tissue is pancreatic tissue, said
30 cells are islets and said enzyme preparation is collagenase.

Fig.1.

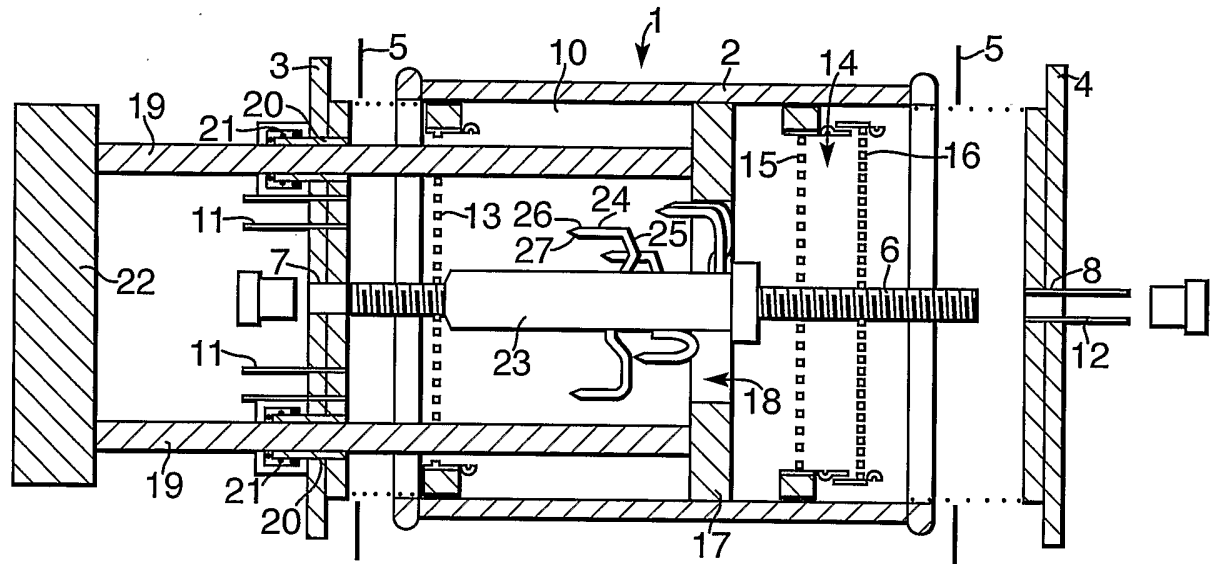


Fig.2.

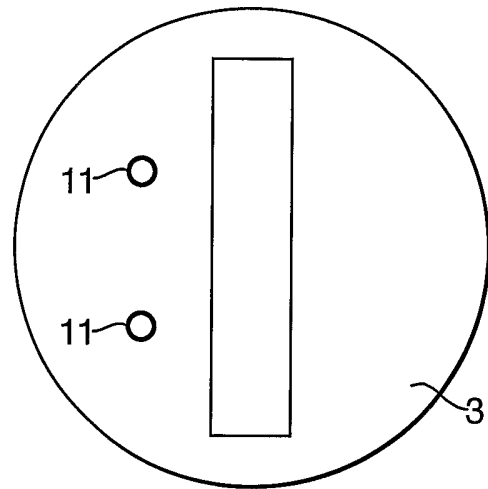


Fig.3.

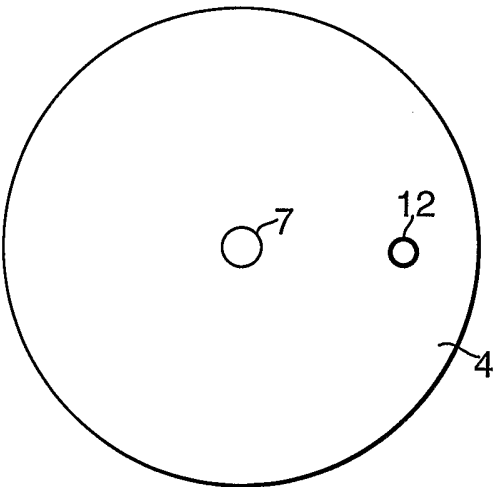


Fig.4.

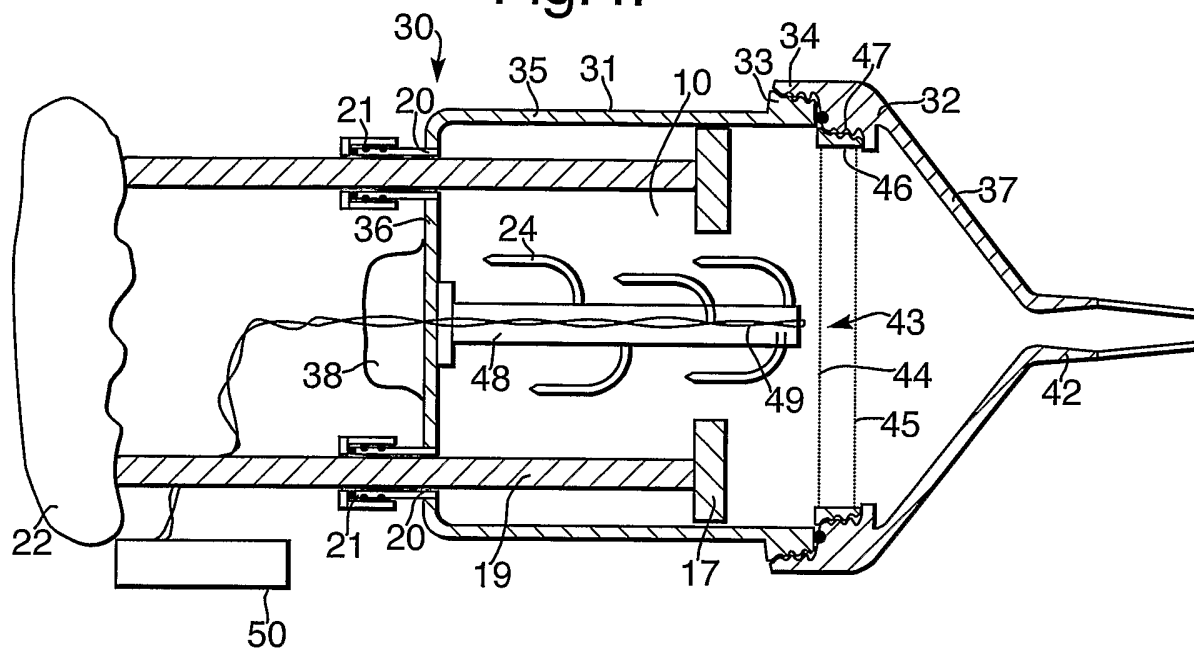


Fig.5.

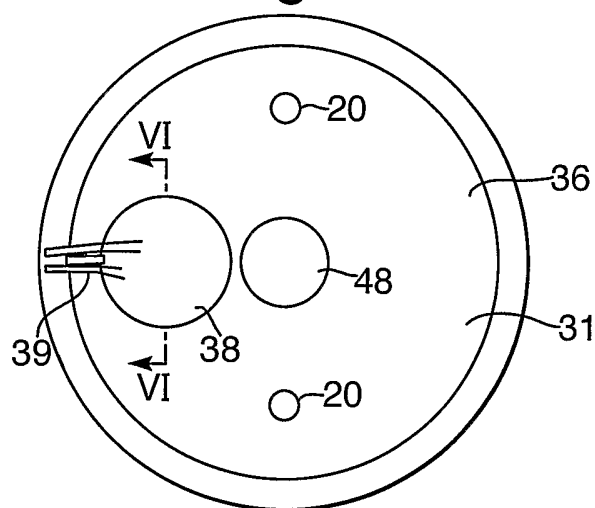
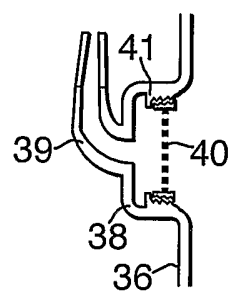


Fig.6.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/002270

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12M3/08

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)
IPC 7 C12M C12N B01F

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, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 786 207 A (KATZ ADAM J ET AL) 28 July 1998 (1998-07-28) column 7, line 11 - line 34 column 9, line 35 - column 10, line 12 column 11, line 48 - line 65; figures 1-4	1-20
Y	US 5 921 477 A (WILLIAMS JOHN G K ET AL) 13 July 1999 (1999-07-13) claim 1; figure 3	1-20
A	PATENT ABSTRACTS OF JAPAN vol. 0122, no. 13 (C-505), 17 June 1988 (1988-06-17) & JP 63 012332 A (SEIKO INSTR & ELECTRONICS LTD), 19 January 1988 (1988-01-19) abstract	1-20
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- * & * document member of the same patent family

Date of the actual completion of the international search

7 September 2004

Date of mailing of the international search report

14/09/2004

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/002270

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 0150, no. 56 (C-0804), 8 February 1991 (1991-02-08) & JP 2 286075 A (NAASARII TECHNOL:KK), 26 November 1990 (1990-11-26) abstract -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB2004/002270

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