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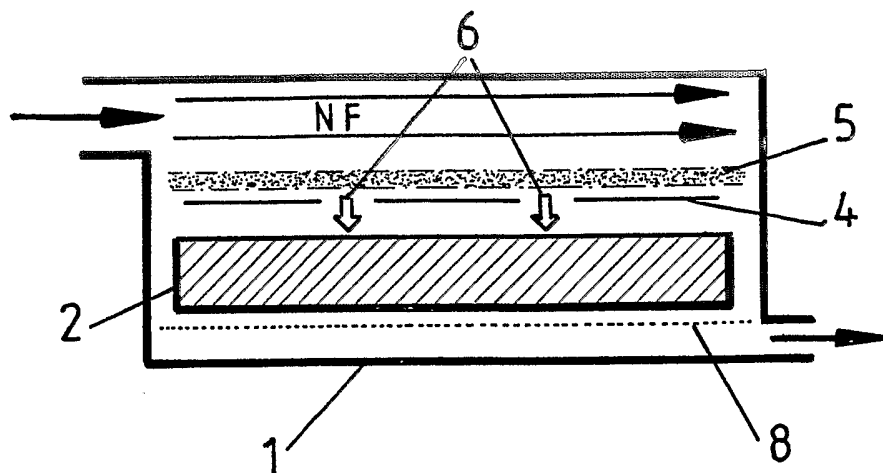
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(54) Title: METHOD AND DEVICE FOR CULTURING TISSUE



(57) Abstract: A method and device for culturing tissue, in particular microvascularized tissue, to aid tissue graft survival by establishing a network of small vessels that can be linked to the host arterial and venous supplies. A scaffold (2) seeded with the cells destined to be the tissue is provided with an upper guide layer (4) having a pattern of apertures (6) therethrough and vascular endothelial cells (5) are delivered to the layer. The scaffold is provided with a chemoattractant for the vascular endothelial cells, such as vascular endothelial growth factor, and fluid is passed over the guide layer. The apertures allow fluid and vascular endothelial cells to migrate into the scaffold only at these focal points to generate capillary sprouts, the migration of cells being stimulated by the chemoattractant within the scaffold.

Title: Method and Device for Culturing Tissue.

DESCRIPTION

The present invention relates to a method and device for culturing tissue, particularly but not exclusively to a method and device for culturing microvascularized tissue.

Replacement cells may be cultivated *in vitro* by taking cells from a patient and allowing them to grow on a two-dimensional surface by supplying the cells with the necessary nutrients for growth. However, in order to produce viable replacement tissue, the cultured tissue must not only be comprised of the same types of cells as the tissue it is to replace but must also have the required three-dimensional shape. Cells grown on a substrate in two-dimensional systems are obliged to initially form monolayers and only the upper surface of cells is available for interaction with the environment and interaction with other cells is difficult. The growth of a three-dimensional tissue construct is achieved by seeding the cells on to a three-dimensional scaffold, for example of collagen, within a bioreactor and passing culture medium through the scaffold.

The fabrication of a tissue that is satisfactory for replacement of damaged/diseased organs, for example a kidney or liver, further requires the fabricated organ to be built around, contain or establish a vascular network. Successful functioning of the fabricated organs requires graft survival to be established immediately after transplantation through the creation of a network of

small vessels which can be linked to the host arterial and venous supplies. Without this, natural vascularisation is not optimal, for example the process may be too slow and/or incomplete to ensure graft survival. It is therefore important that any fabricated tissue or organ is built around or contains or otherwise provides for an established vascular network. To date, tissue engineering systems have not addressed the problem of the requirement for a microvascular supply to enable tissue or organ self-assembly.

It is an object of the present invention to provide a method and device for culturing tissue that aims to overcome, or at least alleviate, the abovementioned drawbacks.

Accordingly, a first aspect of the present invention provides a method for culturing tissue, particularly but not exclusively a microvascularized tissue, comprising seeding a support structure with cells destined to be the tissue and guiding vascular cells and culture medium fluid into the structure at locations along a surface thereof.

A second aspect of the present invention provides a device for culturing tissue, more particularly a microvascularized tissue, the device comprising a housing defining a reaction chamber having an inlet and an outlet for culture medium fluid flow therethrough, a support structure seeded with cells destined to be the tissue located within the chamber and a guide layer provided on a surface of the support structure having one or more apertures therein for guiding the flow of fluid and migration of cells through the layer into the support structure.

Preferably, the guide layer is provided with cell attachment sites on an opposing surface to the support structure. More preferably, the opposing surface of the guide layer is seeded with vascular endothelial cells (VECs). However, other cells may be seeded on to the guide layer depending upon the tissue being cultured. These cells may be provided on the layer prior to its incorporation into the chamber or may be delivered to the layer after its incorporation into the chamber. In this manner, VECs are guided into the support structure via the apertures provided in the guide layer to form microvessels. The layer is preferably comprised of silicon. Any size and pattern of apertures may be provided in the layer depending upon the distribution of microvessels required in the cultured tissue. Preferably, the apertures are 50 - 500 μ m in diameter.

The support structure comprises a scaffold for providing a three-dimensional porous block seeded with cells destined to be the required tissue. The scaffold may be shaped to provide growth of the tissue into the required three-dimensional form or may be a block to form modular constructs that may be fused together to form larger tissue/organs. A number of different scaffold types may be used, such as collagen or polylactic acid. However, collagen is the scaffold of choice.

Preferably, the scaffold is seeded with vascular smooth muscle cells (VSMCs) for surrounding the VECs following their migration into the support structure. In an alternative configuration, the guide layer is first seeded with VSMCs followed by VECs in a ratio determined whereby endothelial cell migration through the apertures

precedes that of VSMCs thus resulting in endothelial cell tubes invested on the outside with smooth muscle cells. In another configuration, the VSMCs may be delivered to the guide layer with the VECs.

Furthermore, the support structure is preferably impregnated with a chemoattractant to attract the VECs into the structure via the apertures in the guide layer. Preferably, the chemoattractant is an angiogenic moiety, more preferably vascular endothelial growth factor (VEGF) or a cocktail of appropriate factors that will encourage vascularisation. In this respect, it is to be appreciated that other biologically active molecules may be provided within the structure to enhance migration of the VECs into the scaffold. The purified chemoattractant itself may be impregnated into the structure or alternatively, unmodified or genetically modified cells that produce the attractant may be provided within the scaffold. These cells may be those which make the tissue itself or may be accessory cells.

The device should preferably be provided with a microporous membrane on a surface of the support structure remote from the guide layer to retain the structure and allow fluid to drain therefrom. Preferably, the guide layer is provided above the support structure and the membrane is provided below.

Thus, the apertures in the guide layer deliver VECs to the support structure in the form of capillary sprouts migrating through the apertures induced by the action of the chemoattractant, and whose distribution is determined by the pattern of apertures. The capillary sprouts are then invested with smooth muscle cells derived from the

support structure or guide layer. The guide layer directs both cells and nutrients into the support structure with the apertures acting as chemoattractant islands for everything within the support structure since the apertures form its only contact areas with the nutrient environment. The apertures also act as focal areas where cell growth is not contact-inhibited with cells being able to migrate downwards into the support structure. The presence of a pressure differential through the structure further stimulates ingrowth and mechanical conditioning.

The fluid inlet and outlet for the chamber are preferably staggered whereby fluid is delivered to the guide layer, passes through the apertures and infiltrates down through the support structure and membrane to exit through the outlet. In this manner, a pulsatile mechanical force is provided by the fluid. The fluid medium may be renewed or recycled.

It is to be appreciated that the guide layer may be made of any material that is capable of having small apertures provided therein and which is suitable for attaching surface bioactive molecules that are known to increase cell attachment and growth. Additional surface bioactive molecules may be adsorbed onto the guide layer surface to enhance cell attachment and growth. Different functional groups may also be provided on the surface of the guide layer to selectively control ECM attachment.

The device of the present invention is preferably incorporated into a bioreactor. To this end, a third aspect of the present invention provides a bioreactor incorporating a device comprising a housing defining a reaction chamber having an

inlet and an outlet for fluid flow therethrough, a support structure seeded with cells destined to be the tissue located within the chamber and a guide layer provided on a surface of the support structure having one or more apertures therein for guiding the migration of cells and flow of fluid through the layer into the support structure.

The bioreactor preferably provides a pulsatile flow of fluid to the chamber. A pulsatile flow further stimulates ingrowth and mechanical conditioning of the tissue culture. More preferably, the bioreactor is one that may be sterilised and which provides optimal conditions for tissue culture, such as being an in-tact system, free from infection and leaks and made of non-toxic materials.

A further possibility of the present invention is to provide small blocks of fabricated tissue which may then be put together. Thicker organs may be generated by multiple layering of support structures, each with its own upper and lower layers in a multiply-configured reactor.

The present invention is particularly suitable for the generation of vascularised tissue. However, it may also be used for the generation of a tubular structure within a tissue matrix, for example for generating collecting ducts (e.g. kidney) or secretory ducts (e.g. liver).

Furthermore, the cells employed in the method may be stem cells wherein the support structure delivers differentiation signals to these cells. The cells may be genetically modified.

The invention will now be illustrated, by way of example only, with reference to the accompanying drawings in which:

Figure 1 is a schematic diagram of a biomodule for the fabrication of micro-vascularised engineered tissue according to one embodiment of the present invention;

Figure 2 is a schematic diagram of a bioreactor fitted with the biomodule shown in Figure 1; and

Figures 3A to 3E schematically illustrate the formation of arterioles using a biomodule according to a second embodiment of the present invention.

The present invention provides a method and device for fabricating micro-vascularised engineered tissue. In a preferred embodiment, a microvascular network is formed within a three-dimensional scaffold using human vascular endothelial and smooth muscle cells, as shown in Figure 1 of the accompanying drawings. A three-dimensional support block of a permeable scaffold or gel 2, for example of collagen, is seeded with cells destined to form the desired organ/tissue. The block is impregnated with Vascular Endothelial Growth Factor (VEGF) to act as an inducer/chemoattractant for angiogenesis. The block 2 is sandwiched between an upper silicon wafer 4 and a microporous membrane in the form of a submicron diffusion mesh 8. The silicon wafer 4 is provided with a pattern of apertures 6 therethrough which were drilled using laser or equivalent technology. The upper surface 5 of the silicon wafer 4 is seeded with Vascular Endothelial Cells (VEC) or a mixture of VEC and Vascular Smooth Muscle Cells (VSMC). VEC, VSMC and VEC

are currently routinely isolated and grown and are commercially available. The sandwich forms a biomodule 1 which is then placed in a bioreactor 10 (see Figure 2) and provided with a pulsatile nutrient fluid flow N.F. across the upper VEC or VEC/VSMC- seeded wafer with suitable temperature and growth conditions.

The VECs grow over the surface of the silicon wafer and are attracted into the block 2 through the apertures 6 that act as guide channels, thus forming capillary sprouts, driven by the VEGF content of the block. This is followed by the migration of VSMC into the block 2 by the direct tracking of VSMC with vascular endothelial cells, as occurs naturally. The microporous membrane 8 retains the scaffold and acts as a drainage surface for the perfused fluid. Once entering the block, the endothelial microtubes are further stimulated to proliferate by the VEGF and to bring in nutrients from the nutrient layer above the silicon wafer 4. Use of the mixed VEC/VSMC cells provides for self-organisation of vessels with a smooth muscle wall. The apertures 6 also allow the introduction of perfusion fluid into the block and provide a pulsatile mechanical force which is important in maintaining the integrity of the microvessels. The conduction of nutrients into the block 2 by the vessels mimics the normal mechanism for organ nutrition, and thus facilitates the self-assembly of complete organs with an intrinsic micro-vascular supply.

The abovementioned production of microvessels within an organ block provides means whereby the natural process for new capillary growth may be mimicked *in vitro*. Capillary growth is known to consist of endothelial cells “budding” out from an established capillary. This outgrowth is tubular and is later

followed by migration of smooth muscle cells which surround the endothelial tube and by a continued process of proliferation eventually form a laminated muscular layer together with synthesis of essential intercellular matrix. The stimuli leading to capillary growth involve secretion of angiogenic factors such as VEGF and matrix-solubilising enzymes such as MMP's, mechanical stress and oxygen tension. The biomodule of the present invention enables a defined microvascular net to be produced, the engineered silicon wafer guide profile determining the distribution and geometry of the net. Furthermore, the incorporation of VEGF or other appropriate growth factors into the support block acts as a specific inducer of capillary sprouting through the guides. The supply of fluid to the upper surface of the guides, rather than around the entire block, creates a differential pressure which encourages endothelial cells to migrate into the support block. The perfusion of fluid into the block is limited to the apertures, thereby creating preferential sites for cell and medium channel formation.

The wafer guide may be comprised of a material other than silicon provided the material is capable of providing small apertures therein (generally, 50-500 μ m in diameter) and is suitable for attaching surface bioactive molecules that are known to increase cell attachment and growth, such as VECs and VSMCs. Any appropriate method may be used to coat the upper layer to provide such a cell attachment surface. The wafer may be seeded with VEC at any time during tissue construction, more preferably being seeded after sufficient cell growth in the tissue block has been established. The VEC and VSMC may be added sequentially or as a mixture. Additional surface bioactive molecules may be adsorbed onto the wafer surface to

enhance cell attachment and growth. A further possibility is to oxidise the silicon wafer surface to improve surface compatibility, for example attaching different functional groups onto the silicon wafers to selectively control ECM attachment.

A suitable bioreactor 10 for incorporation of a biomodule 1 according to the present invention is illustrated in Figure 2. Nutrient fluid is pumped into the biomodule by means of a peristaltic pump 12 from a medium reservoir 14 surrounded by a water jacket 16. The medium reservoir is provided with a gas inlet 18 and a non-return gas valve 20. A small integral heating element 18 fits into the bioreactor which is regulated by an external commercially available thermocouple probe (not shown). As can be seen, nutrient fluid flows into the biomodule over the upper surface of the silicon wafer 4 and then permeates through the support block 2 and mesh 8 and is recycled back to the reservoir. This is in contrast to conventional bioreactor systems that totally surround the organ scaffold with the fluid.

Figures 3A to 3E of the accompanying drawings illustrate the formation of arterioles using a biomodule according to a second embodiment of the present invention. The device is essentially the same as that described in relation to Figures 1 and 2 and accordingly, identical features to those shown in Figures 1 and 2 are given the same reference signs. The porous membrane has also been omitted from the drawings for the sake of simplicity but should be present. Rather than the VSMCs being seeded on the guide layer as shown in the previous embodiment, the VSMCs are provided within the support block 2 along with VEGF, as shown in Figure 3A.

VECs are then seeded onto the guide layer 4, as shown in Figure 3B and the VEGF acts as a chemoattractant 22 to encourage the VECs to migrate into the support structure 2 via the apertures 6 (see Figure 3C), being assisted by the pulsatile pressure P.P. of the nutrient flow N.F. The VECs form capillary sprouts 24 and the VSMCs within the support structure migrate to and surround these tubes to form arterioles, as shown in Figure 3E.

It is to be appreciated that primary seeding may be with VEC or VSMC and VEC, or first with VSMC followed by VEC. In the former, the process relies on self-assortment of VEC and VSMC, whereas with the latter, the VSMC first form capillaries made of VSMC (not VEC) which then become endothelialised.

The examples shown use a collagen scaffold. This is the preferred material of the scaffold in that it is a major component of the extracellular matrix and is compatible with almost all cell types. Furthermore, it is widely available, easily manipulated and readily shaped to provide a scaffold which is morphologically close to the shape of the tissue to be fabricated. Cells or biologically active molecules may be seeded into the scaffold. However, the scaffold may be any material compatible with tissue engineering, such as polyglycolic acid (PGA) or polylactic acid (PLA) based scaffolds.

The angiogenic factors provided within the block, such as VEGF, may be provided by impregnating or adsorbing the purified agent into the block or by

providing unmodified or genetically modified cells that produce the agent within the block, whether being the cells which make the tissue itself or accessory cells.

It is to be appreciated that the cells of the organ block may be any type and configuration (e.g. encapsulated or pre-grown) and accordingly, the method and device of the present invention may be used to construct microvascularised tissue which can be used in a variety of configurations, with many different biomaterials and which is applicable to almost any organ fabrication system. Derivation of cells used may be from natural organs or stem cells, which may be undifferentiated, adult or embryonic, for example for the creation of an endocrine organ like a pancreas. In such a situation, the support block is used to deliver differentiation signals to the stem cells.

A further possibility is to provide small tissue constructs using a biomodule according to the present invention which may then be put together. Thicker organs may be generated by the layering of blocks each with its own upper and lower layers in a multi-configured bioreactor.

The provision of a biomodule according to the present invention for incorporating into a bioreactor provides a more efficient process for a more effective graft, with increased likelihood of the replacement tissue being accepted. The device induces endothelialisation in the cultured tissue which is important in providing a viable graft. The amount and distribution of vascularisation within the cell may be varied depending upon the tissue required. The present invention can be applied to

any organ where a microvascular supply leads to organ or tissue self-assembly.

Furthermore, the device may be applicable to the creation of any tubular structure, such as a kidney or liver, where the tubes (capillaries) are not vessels but, for example collecting (eg. kidney) or secretory (eg. liver) ducts.

CLAIMS

1. A device for culturing tissue comprising a housing defining a reaction chamber having an inlet and an outlet for culture medium fluid flow therethrough a support structure seeded with cells destined to be the tissue located within the chamber and a guide layer provided on a surface of the support structure having one or more apertures therein for guiding the flow of fluid and migration of cells through the layer into the support structure.
2. A device as claimed in claim 1 wherein the guide layer has cell attachment sites on an opposing surface to the support structure.
3. A device as claimed in claim 2 wherein the opposing surface of the guide layer is seeded with vascular endothelial cells.
4. A device as claimed in claim 1, 2 or 3 wherein the guide layer is comprised of silicon.
5. A device as claimed in any one of claims 1 to 4 wherein the apertures are 50-500µm in diameter.
6. A device as claimed in any one of claims 1 to 5 wherein the apertures are provided in a pattern destined to determine the configuration of a vascular or tubular network within the tissue being cultured.
7. A device as claimed in any one of claims 3 to 5 wherein the opposing surface of the guide layer is seeded with vascular smooth muscle cells or a mixture of vascular smooth muscle cells and vascular endothelial cells.

8. A device as claimed in any one of the preceding claims wherein the support structure comprises a three-dimensional porous scaffold seeded with cells destined to be the required tissue.
9. A device as claimed in claim 8 wherein the scaffold is seeded with vascular smooth muscle cells.
10. A device as claimed in claim 8 or 9 wherein the scaffold is shaped to provide growth of the tissue into the required three-dimensioned form.
11. A device as claimed in claim 8 or 9 wherein the scaffold is in the form of a rectangular block to form a modular tissue construct.
12. A device as claimed in any one of the preceding claims wherein the support structure comprises collagen or other biomaterial suitable for creating a cellular tissue.
13. A device as claimed in any one of claims 3 to 12 wherein the support structure is impregnated with a chemoattractant to attract vascular endothelial cells into the structure.
14. A device as claimed in claim 13 wherein the chemoattractant is an angiogenic moiety.
15. A device as claimed in claim 14 wherein the angiogenic moiety is vascular endothelial growth factor.
16. A device as claimed in claim 13, 14 or 15 wherein purified chemoattractant is impregnated into the support structure.
17. A device as claimed in claim 13, 14 or 15 wherein unmodified or genetically modified cells that secrete the chemoattractant are provided within the support structure.

18. A device as claimed in any one of the preceding claims further comprising a microporous membrane on a surface of the support structure remote from the guide layer.
19. A device as claimed in claim 18 wherein the guide layer is provided above the support structure and the membrane is provided below the support structure.
20. A device as claimed in any one of the preceding claims wherein the fluid inlet and outlet are staggered whereby fluid is delivered to an upper surface of the guide layer, passes through the apertures to infiltrate the support structure and exit via the outlet.
21. The use of a device as claimed in any one of claims 1 to 20 for the generation of vascularised tissue.
22. The use of a device as claimed in claim 1 or claim 2 for the generation of a tubular structure within a tissue matrix.
23. The use of a device as claimed in claim 22 for the generation of a collecting or secretory duct.
24. A bioreactor for culturing a three-dimensional tissue, the bioreactor incorporating a device as claimed in any one of claims 1 to 20.
25. A method for culturing tissue comprising seeding a support structure with cells destined to be the tissue and guiding cells and culture medium fluid into the structure at locations along a surface thereof.
26. A method as claimed in claim 25 further comprising laying a guide layer provided with at least one aperture therein on the support structure to guide fluid and cells through the structure.

27. A method as claimed in claim 26 further comprising seeding the guide layer with vascular endothelial cells.
28. A method as claimed in claim 27 further comprising seeding the guide layer with smooth vascular muscle cells.
29. A method as claimed in claim 28 wherein the smooth vascular muscle cells are introduced to the guide layer after introduction of the vascular endothelial cells.
30. A method as claimed in claim 28 wherein the smooth vascular muscle cells are introduced to the guide layer with the vascular endothelial cells.
31. A method as claimed in any one of claims 25 to 27 further comprising seeding the support structure with vascular smooth muscle cells.
32. A method as claimed in any one of claims 25 to 31 further comprising impregnating the support structure with a chemoattractant.
33. A method as claimed in claim 32 wherein the chemoattractant is an angiogenic moiety.
34. A method as claimed in claim 33 wherein the angiogenic moiety is vascular endothelial growth factor.
35. A method as claimed in claim 32, 33 or 34 wherein the support structure is impregnated with purified chemoattractant.
36. A method as claimed in claim 32, 33 or 34 wherein chemoattractant – secreting cells are provided within the support structure.
37. A method as claimed in any one of claims 25 to 36 wherein the cells are stem cells.
38. A method as claimed in claim 37 wherein the support structure delivers differentiation signals to the stem cells.

39. A method as claimed in claim 37 or claim 38 wherein the migration of vascular cells induces differentiation of stem cells.
40. A method as claimed in any one of claims 25 to 39 wherein the cells are genetically modified.
41. A method as claimed in any one of claims 25 to 40 for the generation of vascularised tissue.
42. A method as claimed in claim 25 or claim 26 for the generation of a tubular structure within a tissue matrix.
43. A method as claimed in claim 42 for the generation of a secretory or collecting duct.

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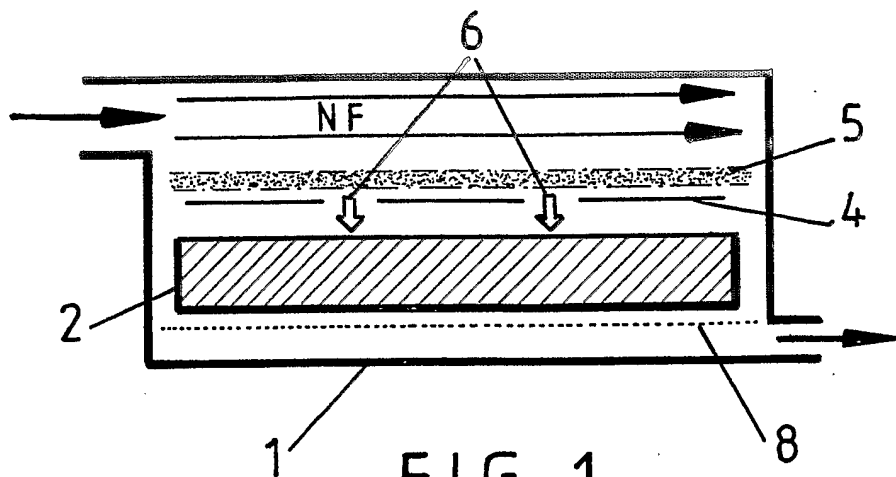


FIG. 1

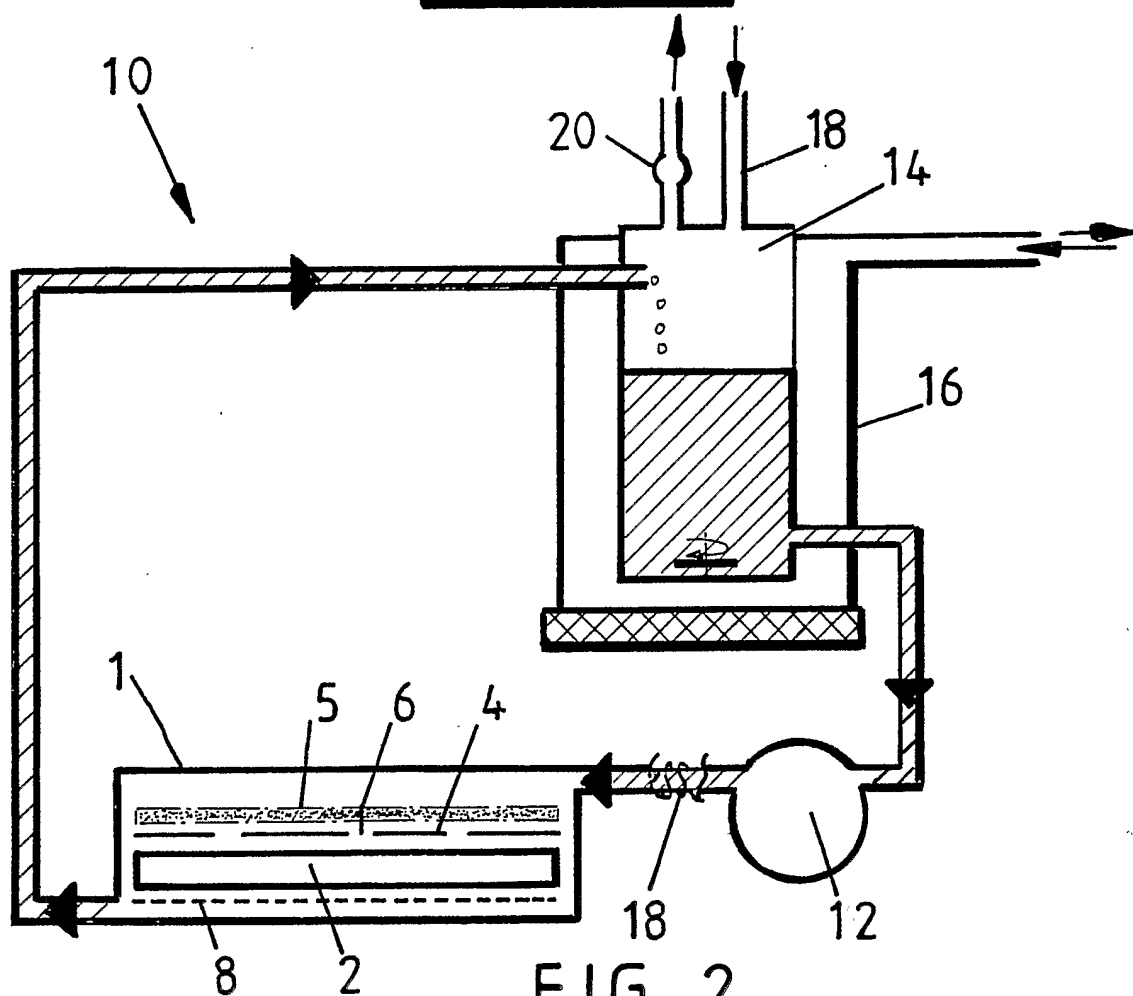


FIG. 2

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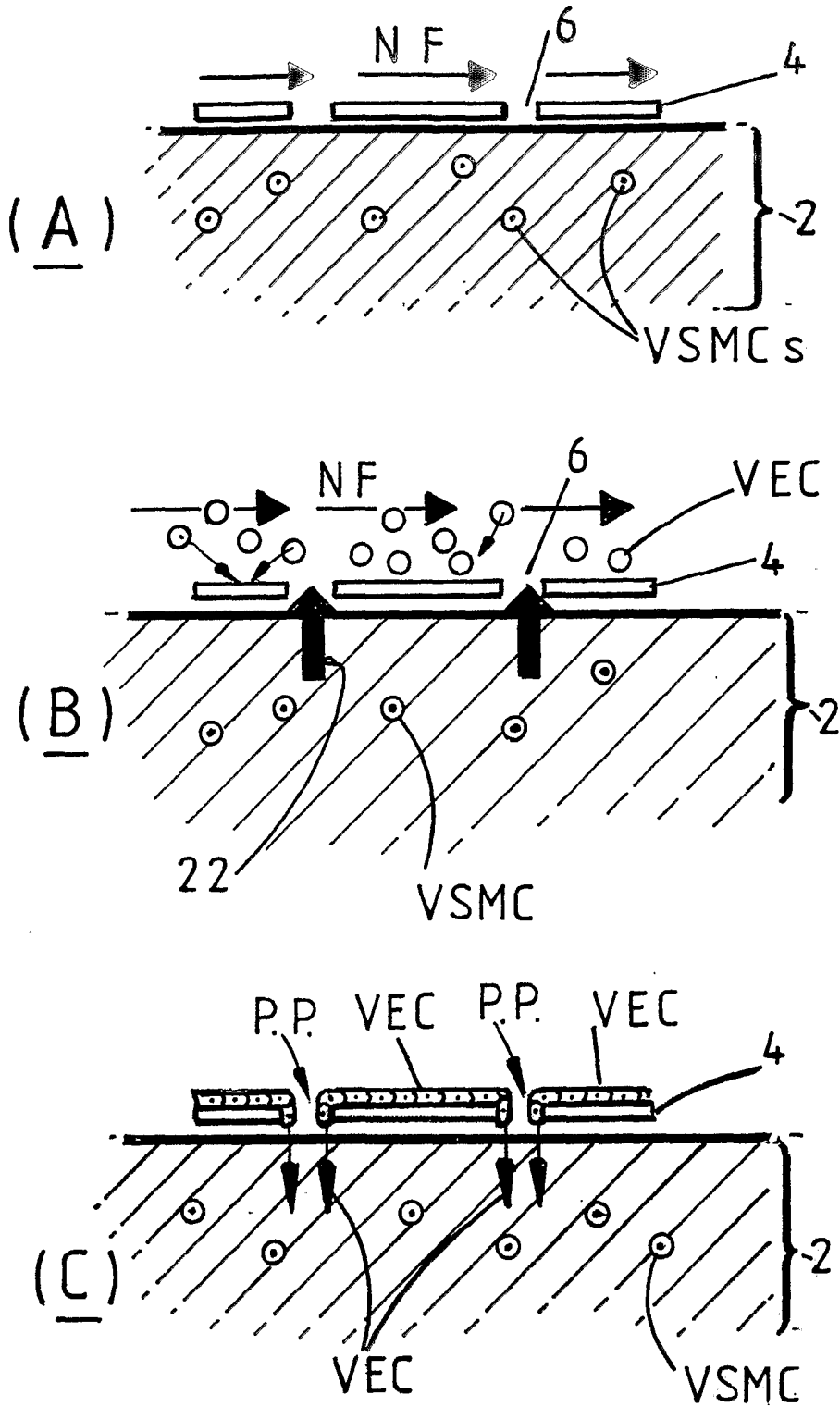


FIG. 3

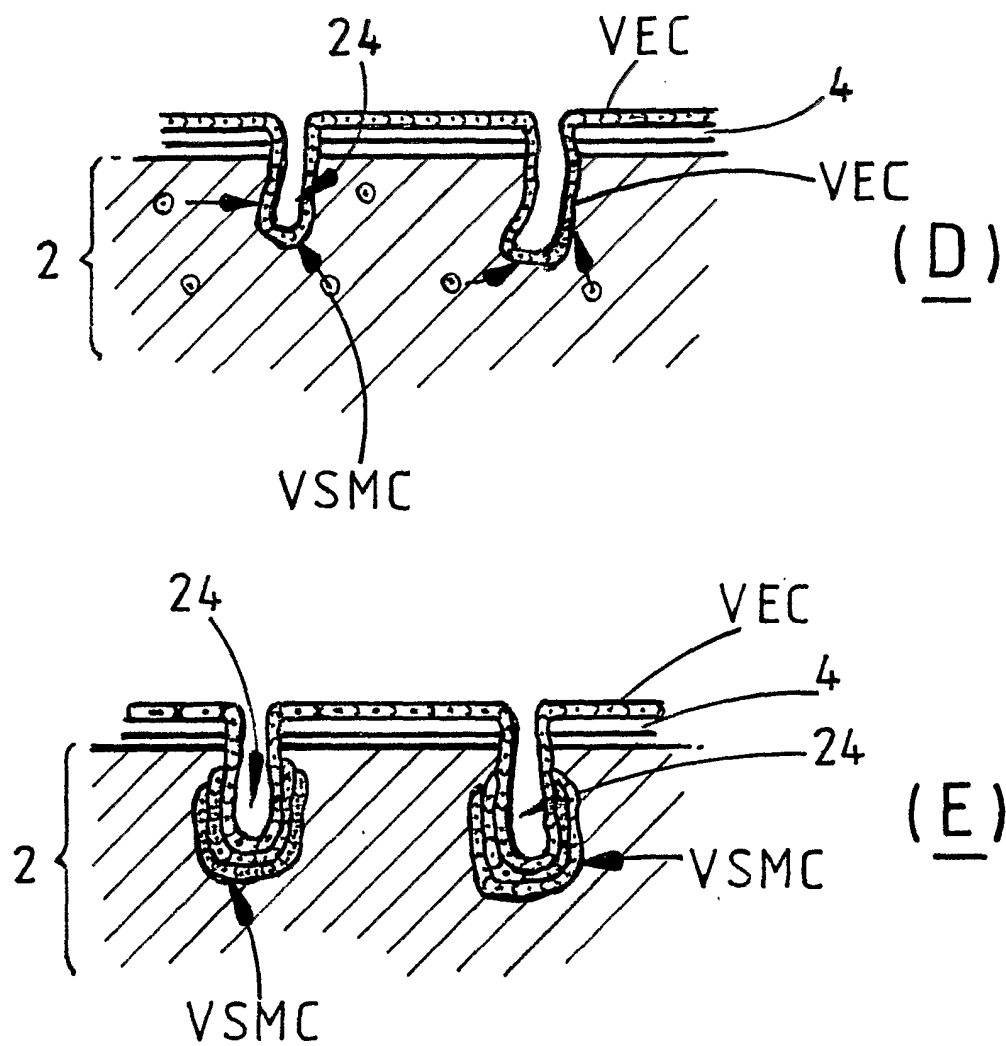


FIG. 3 cont.