



(51) International Patent Classification:

A61F 2/10 (2006.01) *B29C 33/00* (2006.01)
A61L 27/60 (2006.01) *A61L 27/38* (2006.01)
B29C 33/40 (2006.01)

(21) International Application Number:

PCT/EP2018/064371

(22) International Filing Date:

31 May 2018 (31.05.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

17173807.3 31 May 2017 (31.05.2017) EP

(71) Applicant: **ETH ZÜRICH** [CH/CH]; Rämistrasse 101,
8092 Zürich (CH).

(72) Inventors: **FOX, Stephan**; Laufenrietstr. 9, 8608 Bubikon
(CH). **HU, Jimmy**; Feldhof 11, 6300 Zug (CH).

(74) Agent: **SCHMITZ, Joseph**; c/o Isler & Pedrazzini AG,
Postfach 1772, 8027 Zürich (CH).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(54) Title: ASSEMBLY FOR THE PRODUCTION OF TISSUE IMPLANTS

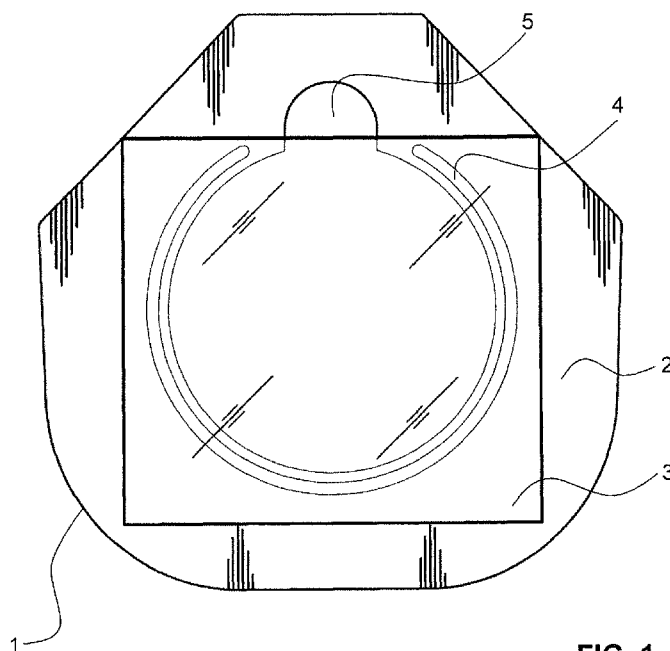


FIG. 1

(57) Abstract: An injection moulding assembly for the production of tissue implants comprising an inner mould element (1) having a mould cavity and an outer mould element (6) having a restriction cavity, the tissue implant comprising at least a first volume of a first tissue equivalent wherein the inner mould element comprises at least one membrane (3) and the mould cavity of the inner mould element is at least partially delimited by said at least one membrane; wherein the inner mould element comprises a gate opening (10) allowing the injection of a first tissue equivalent precursor into the mould cavity; whereby the outer mould element is configured such as to receive the inner mould element within the restriction cavity and the restriction cavity comprises a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity and the at least one membrane of the inner mould element is sealed to the inner mould such as to provide a peelable





(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

TITLE

ASSEMBLY FOR THE PRODUCTION OF TISSUE IMPLANTS

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TECHNICAL FIELD

The present invention relates to an assembly for the production of tissue implants via an injection moulding process.

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PRIOR ART

In today's clinical reality, the quality of tissue implants is determined by the know-how and skills of the person producing such grafts. It is a first challenge to standardize the production process in order to produce tissue implants of a reproducible quality. It is a second challenge to provide an easy-to-use production process requiring a lesser degree of expertise from the operator at equivalent quality when compared to expert manual production. Finally, it is a third challenge to effectively and quickly produce tissue implants.

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A possible approach to these challenges is the production of tissue implants using 3D bio-printing techniques which employ bio-inks that are deposited layer by layer in order to form tissue implants or parts thereof having a certain morphology and/or composition. 3D bio-printing techniques offer the advantage to produce complex 3D structures in an automated fashion and are being pursued for the purposes of applications such as skin graft production. However, the 3D bio-printing techniques inherently suffer from the drawback that they are comparatively slow, since the 3D bio-printer must produce the tissue implants layer by layer.

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In the case of skin implants, one of the best known methods for producing a dermal tissue equivalent, i.e. an implant capable of substituting the dermal layer of the skin, involves using collagen as a matrix material for fibroblasts, since collagen is the main component of

natural skin. In a self-assembly method provided by Bell et al., a dilute collagen hydrogel precursor is mixed with fibroblasts and contracted by the cells themselves. As a result of contraction, excess fluid is squeezed out of the collagen hydrogel and a suitable consistency is attained. However, this method suffers from the drawback that the obtained
5 dermal equivalent is only a fraction in size of the original and thus on one hand, it is impractical since very large precursor collagen hydrogels must be cast in order to reach a contracted graft having useful dimensions and on the other hand the contraction of the precursor hydrogels requires unpractical amounts of time. Additionally, the shrinkage in each direction is not necessarily the same and so the form of the obtained dermal
10 equivalent may undesirably differ from the original form of the precursor collagen hydrogels. Also, the above-described method requires extensive manipulation and is thus prone to contamination in general.

In yet another method which was more recently developed, a dilute hydrogel is first
15 prepared by mixing a dilute collagen solution with a cell suspension and then mechanically compressed using an adapted device to squeeze out excessive fluid and thus arrive at a dermal equivalent which has optimal cell density and mechanical properties suitable for handling during surgical procedure on a human patient. The compaction method reduces manufacturing time to minutes instead of days or weeks when compared to the self-
20 assembly method. However, this method suffers from the drawback that the cells are not homogeneously distributed throughout the bulk, in particular in direction of the compression, of the dermal equivalent. Moreover, the production of more complex implants having different parts as to the type of cells becomes nearly impossible.

25 It is therefore desirable to provide a robust method for producing skin grafts, or dermal equivalents, which allows for a quicker production thereof independently from their three-dimensional structure and which dispenses with the need to reduce water content of an otherwise mechanically fragile dermal equivalent.

SUMMARY OF THE INVENTION

The present invention provides for an apparatus and a process by which tissue implants in general, and skin implants in general, may be provided without having to cast very large precursor collagen hydrogels, without having to rely heavily on skill of the operator and do not warp (do not deform unwantedly) after casting them. In addition, the apparatus and the process according to the present invention allows for increased production speed of tissue implants while the tissue implants display excellent spatial homogeneity, i.e. the cells therein are spatially distributed in an even manner in any direction

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It is an object of the present invention to provide an injection mould assembly for the production of tissue implants comprising an inner mould element having a mould cavity and an outer mould element having a restriction cavity, the tissue implant comprising at least a first volume of a first tissue equivalent wherein the inner mould element comprises at least one membrane and the mould cavity of the inner mould element is at least partially delimited by said at least one membrane; wherein the inner mould element comprises a gate opening allowing the injection of a first tissue equivalent precursor into the mould cavity; characterized in that the outer mould element is configured such as to receive the inner mould element within the restriction cavity and the restriction cavity comprises a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity.

It is a further object of the present invention to provide a process for the production of tissue implants by injection moulding using an injection mould assembly comprising an inner mould element having a mould cavity and an outer mould element having a restriction cavity, the tissue implant comprising at least one volume of a first tissue equivalent, said process comprising the steps of:

- a. bringing the inner mould element and outer mould element together such as to receive the inner mould element within the restriction cavity of the outer mould element;
- b. injecting a first tissue equivalent precursor into the mould cavity of the inner mould element via a gate opening of the inner mould element;
- c. allowing the first tissue equivalent precursor to solidify in the mould cavity of the

inner mould element such as to form an tissue implant comprising a first volume of a first tissue equivalent;

- d. removing the inner mould element from the outer mould element
- e. isolating the tissue implant from the mould cavity of the inner mould element,

5 characterized in that the outer mould element is configured such as to receive the inner mould element within the restriction cavity and the restriction cavity comprises a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity.

10 It is yet a further object of the present invention to provide a tissue implant obtained through the above process according to the second aspect, wherein the tissue implant comprises at least a first and preferably a second volume of tissue equivalent.

It is a another object of the present invention to provide a process for the production of an
15 tissue implant comprising animal cells dispersed in a hydrogel matrix, comprising the steps of:

- a. providing a cross-linkable hydrogel matrix precursor solution in a first storage compartment,
- b. providing a suspension of animal cells in a second storage compartment,
- 20 c. injecting a first tissue equivalent precursor comprised of a mixture of at least the cross-linkable hydrogel matrix precursor solution and the suspension of animal cells into a mould cavity of a mould, such as the above-mentioned mould cavity of the inner mould element,
- d. allowing the first tissue equivalent precursor to solidify in the mould cavity such as
25 to form a tissue implant comprising a first volume of a first tissue equivalent in the mould cavity of the mould, wherein the first tissue equivalent precursor comprised of a mixture of at least the cross-linkable hydrogel matrix precursor solution and the suspension of animal cells is formed by combining at least a separate flow of cross-linkable hydrogel matrix precursor solution and at least a separate flow of suspension of animal cells in a mixing
30 cavity located downstream of the storage compartments and upstream of the mould cavity of the mould and fluidly connected with each storage compartment and wherein the cross-linkable hydrogel matrix precursor solution is a concentrated cross-linkable hydrogel matrix precursor solution.

Further embodiments of the invention are laid down in the dependent claims.

BRIEF DESCRIPTION OF THE DRAWINGS

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Preferred embodiments of the invention are described in the following with reference to the drawings, which are for the purpose of illustrating the present preferred embodiments of the invention and not for the purpose of limiting the same. In the drawings,

- 10 Fig. 1 shows a front view of an inner mould element (1) formed of an annular frame (2) which is delimited on both sides by a membrane (3) of square shape, which membrane is affixed to the frame by a seal (4). The inner mould element further comprises a gate opening (5) through which a tissue equivalent precursor can be injected into the mould cavity.
- 15 Fig. 2 shows a perspective view of the inner mould element shown in Fig.1
- Fig. 3 shows a perspective view of the inner mould element shown in Fig.1, where the membrane (3) has been partially peeled away.
- 20 Fig. 4 shows a perspective view of an injection mould assembly comprising the inner mould element shown in Fig.1 and an outer mould element (6), in which the inner mould element (1) is received in the outer mould element (6). The outer mould element (6) is formed of an upper die (8) formed by a rigid wall and an lower die (7) formed by a rigid wall, where the two dies are connected to each other through the distance adjusting means (9) which are fixed in position. The upper die further comprises a gate opening (10) through which a tissue equivalent precursor can be injected into the mould cavity through the gate opening (5) of the inner mould element (1).
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- 30 Fig. 5 shows a perspective view of an injection mould assembly comprising an inner mould element (1) and an outer mould element (6). The inner mould element (1) is formed of a rectangular frame (2) which is delimited on its upper side by a membrane (3) of square shape, which membrane is affixed

to the frame by a seal. The inner mould element further comprises a gate opening (5) through which a tissue equivalent precursor can be injected into the mould cavity. The inner mould element (1) can be inserted laterally into the outer mould element (6). The outer mould element (6) further comprises a gate opening (10).

Fig. 6 shows a perspective view of an injection mould assembly in which the inner mould element (1) is received in the outer mould element (6).

Fig. 7 shows a cross-sectional view of an injection mould assembly comprising the inner mould element shown in Fig.1 and an outer mould element (6), in which the inner mould element (1) is received in the outer mould element (6). The outer mould element (6) is formed of an upper die (8) formed by a rigid wall and an lower die (7) formed by a rigid wall, where the two dies are connected to each other through the distance adjusting means (9) which are fixed in a position where the upper die restricts the outward bulging of the membrane (3) during the injection of a first tissue equivalent precursor (11). The upper die further comprises a gate opening (10) through which a tissue equivalent precursor can be injected into the mould cavity through the gate opening (5) of the inner mould element (1).

Fig. 8 shows a cross-sectional view of an injection mould assembly comprising the inner mould element shown in Fig.1 and an outer mould element (6), in which the inner mould element (1) is received in the outer mould element (6). The outer mould element (6) is formed of an upper die (8) formed by a rigid wall and an lower die (7) formed by a rigid wall, where the two dies are connected to each other through the distance adjusting means (9) which are first adjusted to a predetermined distance with respect to the membrane (3) and then fixed in a position where the upper die allows the outward bulging of the membrane (3) during the injection of a second tissue equivalent precursor (12). The upper die further comprises a gate opening (10) through which a tissue equivalent precursor can be injected into the mould cavity through the gate opening (5) of the inner mould element (1).

DESCRIPTION OF PREFERRED EMBODIMENTS

Suitable membranes for use in the present invention are permeable or impermeable membranes, and in particular permeable membranes that form a selective barrier through which liquids such as aqueous solutions and/or gases such as carbon dioxide or oxygen can move. Suitable permeable membranes may be porous membrane or meshed screens. Examples of such porous membranes are PET membranes or PTFE membranes, while meshed screens may for example be screens made from polyamide or polyolefins. Examples of impermeable membranes are for example aluminium foil or other barrier films.

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The term “skin” is usually understood as referring to a construct having at least two layers and cell types. The first layer comprises the dermis populated with fibroblasts in an extracellular matrix (ECM) and the second layer comprises the epidermis populated with keratinocytes. The production of the epidermis is based on seeding keratinocytes on top of a dermal equivalent, either by pipetting, robotic application (e.g. 3D bioprinting) or spraying. The dermal equivalent can vary in the composition and fabrication method of the ECM and the integration of the fibroblasts. In dermal equivalents for clinical application, it is important to have a homogenous distribution of vital fibroblasts throughout the bulk of epidermal layer and to have sufficient mechanical stability for handling during surgery.

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It is an object of the present invention to provide an injection mould assembly for the production of tissue implants comprising an inner mould element having a mould cavity and an outer mould element having a restriction cavity, the tissue implant comprising at least a first volume of a first tissue equivalent wherein the inner mould element comprises at least one membrane and the mould cavity of the inner mould element is at least partially delimited by said at least one membrane; wherein the inner mould element comprises a gate opening allowing the injection of a first tissue equivalent precursor into the mould cavity; characterized in that the outer mould element is configured such as to receive the inner mould element within the restriction cavity and the restriction cavity comprises a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the

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restriction cavity. In a preferred embodiment, the rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity essentially abuts against or rests on the outer side of the membrane delimiting the mould cavity. This way, a deformation and in particular bulging of the membrane during the injection of a first volume of a first tissue equivalent precursor is prevented.

In a preferred embodiment, in the injection mould assembly according to the present invention, the rigid wall of the restriction cavity is configured such as to be adjustable with respect to the distance between at least one membrane of the inner mould element and the rigid wall. For example the rigid wall may be connected to distance adjusting means such as actuators that allow controlling the position of the rigid wall. This allows, at least in the region of the membrane delimiting the mould cavity, to adjust the extent to which the membrane bulges outward in response to the pressure of the tissue equivalent precursor being injected. For example, in a first step, the rigid wall may be in a resting position in which the rigid wall essentially rests on the outer side of the membrane during the injection of a first volume of a first tissue equivalent precursor such as to restrict any outward bulging of the membrane due to injection pressure. In a second step, the rigid wall may then be in a position in which the rigid wall is at an increased distance from the membrane during the injection of a further volume of a further tissue equivalent precursor such as to not restrict any outward bulging of the membrane due to injection pressure. This allows the further tissue equivalent precursor to enter between the membrane and the first tissue equivalent precursor and thereby expand the membrane such as to form a second volume of further tissue equivalent on top of, or adjacent to, the first volume of first tissue equivalent. After the injection process ends, i.e. the injection pressure is removed, the elasticity of the membrane pushes back the membrane and gently squeezes the second volume of second tissue equivalent precursor, thereby slowly dewatering it. Thus, in a preferred embodiment, in the injection mould assembly according to the present invention, the at least one membrane of the inner mould element is an elastic membrane, preferably having an elasticity such as to be allow the expansion thereof when an injection pressure is applied and to allow the contraction thereof when the injection pressure is removed.

In a preferred embodiment, in the injection mould assembly according to the present

invention, the at least one membrane of the inner mould element is sealed to the inner mould, and in particular to the closed frame. In another preferred embodiment, in the injection mould assembly according to the present invention, the at least one membrane of the inner mould element is sealed to the inner mould, and in particular to the closed frame, such as to provide a peelable seal. A peelable seal allows to easily peel away the membrane in order to isolate the tissue implant from the inner mould element, much like a lid of a receptacle. This may be achieved according to techniques known to the person of skill in the art, such as for example by providing the membrane with a heat seal layer which can be heat sealed to the inner mould element. The heat seal may for example comprise ethylene-acid copolymers, ionomers or LDPE. Alternative methods to form seals are HF, ultrasound or laser welding. Alternatively, a seal may also be achieved by depositing an adhesive material where a seal is to be formed. In preferred embodiment, the membrane is provided with one or more pull tabs in order to facilitate the peeling away of the membrane. One or more pull tabs may be formed, for example, by providing a rectangular membrane delimiting a circular part of the mould cavity through a circular seal, wherein the rectangular membrane, at least in one direction, extends beyond the circular seal.

The elements of the injection mould assembly for the production of tissue implants may be from any suitable material and in particular may be formed from polymer material.

In a preferred embodiment, in the injection mould assembly according to the present invention, the inner mould element is made from a biocompatible material such as for example a biocompatible polymer. Examples of such a polymer can be polyolefins, polyester, PEEK, styrene copolymers and such. The advantage of using such an inner mould element is that, after solidification of the tissue equivalent precursor and before implantation of the tissue implant, the inner mould element incorporating the solidified tissue equivalent precursor therein may be placed integrally into a cell culturing medium for further cultivation. The membrane of the inner mould element then allows the exchange of nutrients and gases between the cell culturing medium and the cells of the tissue implant. Likewise, the rigid parts of the inner mould element such as for example an annular or rectangular frame allow in general for an easier manipulation of the inner mould element, i.e. the removing of the inner mould element from the restriction cavity of the

outer mould element in order to place the inner mould element into a culturing vessel and offer a hold for when the membrane is peeled away to isolate the tissue implant.

5 In another preferred embodiment, in the injection mould assembly according to the present invention, the inner mould element comprises two membranes facing each other and wherein each membrane is affixed to either side of a closed frame such as to form the mould cavity. While the form of the frame is not limited, the closed frame may for example be of annular or polygonal shape. Examples of polygonal shapes are equiangular polygonal shapes such as triangular, rectangular, square or hexagonal.

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In another preferred embodiment, in the injection mould assembly according to the present invention, the at least one membrane is a porous membrane preferably having pores having a diameter of 0.25 μm or more, and more preferably having a diameter in the range of 0.25 μm to 7.5 μm . The diameters of the pores allow to exchange liquids, nutrients and gases
15 between a cell culturing medium and a tissue implant comprised in the inner mould element.

In another preferred embodiment, in the injection mould assembly according to the present invention, the inner mould element and the restriction cavity of the outer mould element
20 are in positive lock. For example, the outer mould element may consist of two dies such as an upper die and an lower die that can be arranged such as to lock the inner mould in place and form a restriction cavity in positive lock with the inner mould.

In another preferred embodiment, in the injection mould assembly according to the present
25 invention, the inner mould element has a cuboid geometry and at least one face of the cuboid is formed by the at least one membrane and preferably two opposing faces of the cuboid are formed by two membranes, respectively, and the remaining faces are formed by a closed frame having a rectangular or square shape.

30 In another preferred embodiment, in the injection mould assembly according to the present invention, the inner mould element has a cylindrical geometry and at least one face of the cylinder is formed by the at least one membrane and preferably two opposing faces of the cylinder are formed by two membranes, respectively, and the lateral face is formed by an

closed annular frame. In a more preferred embodiment, the inner mould element has a flat cylindrical geometry, i.e. of a disc. In this case, the inner mould element is preferably comprised of a closed annular frame on which two opposing faces of the cylinder are formed by two membranes sealed to each side of the annular frame. The annular frame
5 may for example be formed from a polymer, especially a bio-compatible polymer and the membranes may be formed from a biocompatible polymer as well, such as for example PTFE, PP, PE or PET.

It is a further object of the present invention to provide a process for the production of
10 tissue implants by injection moulding using an injection mould assembly comprising an inner mould element having a mould cavity and an outer mould element having a restriction cavity, the tissue implant comprising at least one volume of a first tissue equivalent, said process comprising the steps of:

- a. bringing the inner mould element and outer mould element together such as to
15 receive the inner mould element within the restriction cavity of the outer mould element;
- b. injecting a first tissue equivalent precursor into the mould cavity of the inner mould element via a gate opening of the inner mould element;
- c. allowing the first tissue equivalent precursor to solidify in the mould cavity of the inner mould element such as to form an tissue implant comprising a first volume of a first
20 tissue equivalent;
- d. removing the inner mould element from the outer mould element
- e. isolating the tissue implant from the mould cavity of the inner mould element,
characterized in that the outer mould element is configured such as to receive the inner
mould element within the restriction cavity and the restriction cavity comprises a rigid wall
25 delimiting at least the part of the restriction cavity facing the membrane delimiting the
mould cavity when the inner mould element is received within the restriction cavity.
During step b. and c., the rigid wall of the outer mould element essentially resting on the
outer side of the membrane restricts an outward bulging of the membrane during the
injection of the first tissue equivalent precursor into the mould cavity by being fixed in this
30 position. Thus, the tissue implant can be formed in a shape that essentially corresponds to
the mould cavity of the inner mould element, such as for example a flat disc shape.

In another preferred embodiment of the process the production of tissue implants by

injection moulding using an injection mould assembly according to the present invention, the rigid wall of the restriction cavity is configured such as to be adjustable with respect to the distance between at least one membrane of the inner mould element and the rigid wall and wherein between steps c. and d., the process further comprises the steps c1 to c3 of

- 5 c1. adjusting the distance between at least one membrane of the inner mould element and the rigid wall such as to increase the distance between the at least one membrane of the inner mould element and the rigid wall,
- c2. injecting a second volume of a second tissue equivalent precursor into the mould cavity of the inner mould, between the at least one membrane of the inner mould element
10 and the first volume of first tissue equivalent;
- c3. allowing the second tissue equivalent precursor to settle or solidify in the mould cavity of the inner mould element such as to form a tissue implant further comprising a second volume of second tissue equivalent adjacent to the first volume of first tissue equivalent. During step c2. and c3., the rigid wall of the outer mould element is fixed at an
15 adjusted distance from the outer side of the membrane and thereby permits an outward bulging of the membrane during the injection of the second tissue equivalent precursor.

It is another alternative object of the present invention to provide a process for the production of tissue implants by injection moulding using an injection mould assembly
20 comprising an inner mould element having a mould cavity and a first and a second outer mould element each having a restriction cavity, the tissue implant comprising at least one volume of a first tissue equivalent and at least one volume of a second tissue equivalent, said process comprising the steps of:

- a. bringing the inner mould element and first outer mould element together such as to
25 receive the inner mould element within the restriction cavity of the first outer mould element and wherein the ;
- b. injecting a first tissue equivalent precursor into the mould cavity of the inner mould element via a gate opening of the inner mould element;
- c. allowing the first tissue equivalent precursor to solidify in the mould cavity of the
30 inner mould element such as to form an tissue implant comprising a first volume of a first tissue equivalent;
- d. bringing the inner mould element comprising a first volume of a first tissue equivalent and the second outer mould element together such as to receive the inner mould element

within the restriction cavity of the second outer mould element;

e. injecting a second tissue equivalent precursor into the mould cavity of the inner mould element via a gate opening of the inner mould element;

f. allowing the second tissue equivalent precursor to settle or solidify in the mould cavity of the inner mould element such as to form an tissue implant comprising a second volume of a second tissue equivalent adjacent to, or on top of, the first volume of a first tissue equivalent;

g. removing the inner mould element from the second outer mould element

h. isolating the tissue implant from the mould cavity of the inner mould element,

10 characterized in that the first and second outer mould elements are configured such as to receive the inner mould element within their respective restriction cavity and the restriction cavities each comprise a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity and wherein the rigid wall of the first outer mould element rests immovably on the outer side of the membrane, thereby restricting an outward bulging of the membrane during the injection of the first tissue equivalent precursor and wherein the rigid wall of the second outer mould element is immovably placed at an increased distance from the outer side of the membrane, thereby permitting an outward bulging of the membrane during the injection of the second tissue equivalent precursor.

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In another preferred embodiment of the process the production of tissue implants by injection moulding using an injection mould assembly according to the present invention, the first tissue equivalent is a dermal equivalent comprising fibroblasts suspended in a hydrogel matrix, wherein preferably the hydrogel matrix is a collagen matrix. In a yet more

25 preferred embodiment of the process the production of tissue implants by injection moulding using an injection mould assembly according to the present invention, the first tissue equivalent is a dermal equivalent comprising fibroblasts suspended in a hydrogel matrix, and in particular is a dermal equivalent comprising fibroblasts suspended in a crosslinked collagen matrix. In this case, the first tissue equivalent is formed by injecting a first tissue equivalent precursor comprising fibroblasts suspended in a collagen solution into the mould cavity of the inner mould element via a gate opening of the inner mould element, wherein preferably the collagen solution has a collagen concentration of at least 10 mg/ml or of from 10 mg/ml to 25 mg/ml, preferably of at least 15 mg/ml or of from 15

mg/ml to 25 mg/ml and more preferably of at least 17.5 mg/ml or of from 17.5 mg/ml to 25 mg/ml and more preferably is a collagen solution of un-pepsinized collagen.

In another preferred embodiment of the process the production of tissue implants by injection moulding using an injection mould assembly according to the present invention, the second tissue equivalent is an epidermal equivalent comprising keratinocytes and preferably is a suspension of keratinocytes in cell culturing medium. In this case, the second tissue equivalent is formed by injecting a second tissue equivalent precursor comprising fibroblasts, preferably suspended in a cell culturing medium, into the mould cavity of the inner mould element via a gate opening of the inner mould element. The second tissue equivalent precursor then accumulates between the membrane and the first tissue equivalent because of the deformation of the membrane due to injection pressure and forms a second tissue equivalent on top of, or adjacent to, the first tissue equivalent thereby yielding a tissue implant comprising two discrete parts which differ in cell type, i.e. the first and second tissue equivalents.

In another preferred embodiment of the process the production of tissue implants by injection moulding using an injection mould assembly according to the present invention, the tissue implant is a skin implant, preferably comprising at least a dermal equivalent comprising fibroblasts in a collagen matrix and an epidermal equivalent comprising keratinocytes.

It is yet a further object of the present invention to provide a tissue implant obtained through the above process according to the second aspect, wherein the tissue implant comprises at least a first and preferably a second volume of tissue equivalent.

It is another object of the present invention to provide a process for the production of an tissue implant comprising animal cells dispersed in a hydrogel matrix, comprising the steps of:

- a. providing a cross-linkable hydrogel matrix precursor solution in a first storage compartment,
- b. providing a suspension of animal cells in a second storage compartment,

- c. injecting a first tissue equivalent precursor comprised of a mixture of at least the cross-linkable hydrogel matrix precursor solution and the suspension of animal cells into a mould cavity of a mould, such as the above-mentioned mould cavity of the inner mould element,
- 5 d. allowing the first tissue equivalent precursor to solidify in the mould cavity such as to form a tissue implant comprising a first volume of a first tissue equivalent in the mould cavity of the mould, wherein the first tissue equivalent precursor comprised of a mixture of at least the cross-linkable hydrogel matrix precursor solution and the suspension of animal cells is formed by combining at least a separate flow of cross-linkable hydrogel matrix precursor solution and at least a separate flow of suspension of animal cells in a mixing
- 10 cavity located downstream of the storage compartments and upstream of the mould cavity of the mould and fluidly connected with each storage compartment and wherein the cross-linkable hydrogel matrix precursor solution is a concentrated cross-linkable hydrogel matrix precursor solution.
- 15 The process for the production of an tissue implant comprising animal cells dispersed in a hydrogel matrix allows for the production of an tissue implant comprising animal cells dispersed in a hydrogel matrix, and is especially useful in the production of tissue implants such as skin grafts because on one hand, the water content of the hydrogel matrices useful in the present invention is beneficial to the tissue regeneration of skin and on the other
- 20 hand, the mechanical properties conferred by the scaffold formed by the hydrogel matrices useful in the present invention render the manipulation of the engineered skin graft uncomplicated during typical surgical procedure in which the graft is applied. By providing a cross-linkable hydrogel matrix precursor solution in a first storage compartment, it is possible to store the fluid solution for a prolonged time until needed for
- 25 the production of the engineered tissue graft and thus to generate a fresh graft on-demand provided the animal cells are in a state where they can be combined with the cross-linkable hydrogel matrix precursor solution. Furthermore, by efficiently combining at least a separate flow of cross-linkable hydrogel matrix precursor solution and at least a separate flow of suspension of animal cells in a mixing cavity, it is possible to achieve efficient
- 30 mixing without having to subject the cells to an amount of shear that would otherwise negatively impact the viability of the animal cells and to essentially shift the onset of cross-linking to when the mixture of at least the cross-linkable hydrogel matrix precursor solution and the suspension of animal cells reaches the mould cavity. Another benefit of

using a concentrated solution of cross-linkable hydrogel matrix precursor is that the immediately resulting tissue graft has the right consistency for use in a surgical procedure.

The cross-linkable hydrogel matrix precursor solutions are preferably aqueous solutions of cross-linkable hydrogel matrix precursor. Such cross-linkable hydrogel matrix precursor includes cross-linkable hydrogel matrix precursors which are cross-linkable by addition of a cross-linking agent, change in pH, change in temperature or irradiation with for example UV. In particular, the cross-linkable hydrogel matrix precursors are either cross-linkable hydrogel matrix precursors which are cross-linkable by addition of a cross-linking agent or by change of pH from for example acidic towards neutral/basic. Exemplary cross-linkable hydrogel matrix precursor may be selected from proteins such as gelatin, collagen, fibrinogen; from polysaccharides such as starch, agarose; from synthetic polymers such as polyacrylamide or poloxamers. A preferred cross-linkable hydrogel matrix precursor solution may be for example be an aqueous solution of collagen preferably having an acidic pH.

The suspension of animal cells may be a suspension of animal cells in a liquid medium such as for example cell culturing medium. For instance, in the case of fibroblasts, the cells may be suspended in DMEM (Dulbecco modified Eagle medium). The concentration of animal cells within the suspension of cells may be chosen such that a animal cell target density is reached in the tissue implant. It will be apparent to a person of ordinary skill in the art that the animal cells are to be chosen depending on the type of tissue graft to be produced. As an example, in the case where the tissue graft is a skin graft, the animal cells may be chosen from fibroblasts and/or keratinocytes.

The suspension of animal cells as well as the cross-linkable hydrogel matrix precursor solution are stored in their respective storage compartments before being combined in a mixing cavity to which they are fluidly connected to form a tissue equivalent precursor. The storage compartments may be present as separate bodies or may be comprised in a single injection moulding apparatus, such as for example a double syringe incorporating the first and second storage compartment. The double syringe may be equipped with plungers that can be independently controlled in order to adjust and maintain the flow rates from the compartments into the mixing cavity and into the mould cavity of the mould, such

as the inner mould element.

When the tissue equivalent precursor comprising the mixture of cross-linkable hydrogel matrix precursor solution and the suspension of animal cells is lead into the mould cavity
5 in order to solidify therein, the solidification may for example be completed within 10 minutes.

The mixture of cross-linkable hydrogel matrix precursor solution and the suspension of animal cells is formed by combining at least a separate flow of cross-linkable hydrogel
10 matrix precursor solution and at least a separate flow of suspension of animal cells in a mixing cavity located downstream of the storage compartments and upstream of the mould cavity of the mould such as the inner mould element and fluidly connected with each storage compartment. The mixing cavity may comprise a mixing element such as a static mixing element which increases the mixing of the two streams. However, the mixing
15 cavity may not comprise a mixing element and rely solely on the mixing effect achieved by the turbulent flow within the mixing cavity.

LIST OF REFERENCE SIGNS

1	inner mould element	8	upper mould element die
2	frame	9	distance adjusting means
3	membrane	10	gate opening
4	seal	11	first volume of tissue equivalent
5	gate opening	12	second volume of tissue equivalent
6	outer mould element		
7	lower mould element die		

CLAIMS

1. An injection moulding assembly for the production of tissue implants comprising an inner mould element having a mould cavity and an outer mould element having a restriction cavity, the tissue implant comprising at least a first volume of a first tissue equivalent
wherein the inner mould element comprises at least one membrane and the mould cavity of the inner mould element is at least partially delimited by said at least one membrane;
wherein the inner mould element comprises a gate opening allowing the injection of a first tissue equivalent precursor into the mould cavity;
characterized in that the outer mould element is configured such as to receive the inner mould element within the restriction cavity and the restriction cavity comprises a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity and the at least one membrane of the inner mould element is sealed to the inner mould such as to provide a peelable seal.
2. The injection mould assembly according to claim 1, wherein the rigid wall of the restriction cavity is configured such as to be adjustable with respect to the distance between at least one membrane of the inner mould element and the rigid wall.
3. The injection mould assembly according to claim 1 or 2, wherein the inner mould element comprises two membranes facing each other and wherein each membrane is affixed to either side of a closed frame such as to form the mould cavity.
4. The injection mould assembly according to any of the preceding claims, wherein the at least one membrane is a porous membrane preferably having pores having a diameter of 0.25 μm or more, and more preferably having a diameter in the range of 0.25 μm to 7.5 μm .
5. The injection mould assembly according to any of the preceding claims, wherein the inner mould element and the restriction cavity of the outer mould

element are in positive lock.

6. The injection mould assembly according to claim 1 or 2, wherein the inner mould element has a cuboid geometry and at least one face of the cuboid is formed by the at least one membrane and preferably two opposing faces of the cuboid are formed by two membranes, respectively.
7. The injection mould assembly according to claim 1 or 2, wherein the inner mould element has a cylindrical geometry and at least one face of the cylinder is formed by the at least one membrane and preferably two opposing faces of the cylinder are formed by two membranes, respectively.
8. A process for the production of tissue implants by injection moulding using an injection moulding assembly comprising an inner mould element having a mould cavity and an outer mould element having a restriction cavity according to any of claims 1 to 7, the tissue implant comprising at least one volume of a first tissue equivalent, said process comprising the steps of:
 - a. bringing the inner mould element and outer mould element together such as to receive the inner mould element within the restriction cavity of the outer mould element;
 - b. injecting a first tissue equivalent precursor into the mould cavity of the inner mould element via a gate opening of the inner mould element;
 - c. allowing the first tissue equivalent precursor to solidify in the mould cavity of the inner mould element such as to form a tissue implant comprising a first volume of a first tissue equivalent;
 - d. removing the inner mould element from the outer mould element
 - e. isolating the tissue implant from the mould cavity of the inner mould element,
characterized in that the outer mould element is configured such as to receive the inner mould element within the restriction cavity and the restriction cavity comprises a rigid wall delimiting at least the part of the restriction cavity facing the membrane delimiting the mould cavity when the inner mould element is received within the restriction cavity.
9. The process for the production of tissue implants by injection moulding using according to claim 8, wherein the rigid wall of the restriction cavity is configured such as to be adjustable with respect to the distance between at

least one membrane of the inner mould element and the rigid wall and wherein between steps c. and d., the process further comprising the steps i to iii of

- i. adjusting the distance between at least one membrane of the inner mould element and the rigid wall such as to increase the distance between the at least one membrane of the inner mould element and the rigid wall,
 - ii. injecting a second volume of a second tissue equivalent precursor in to the mould cavity of the inner mould, between the at least one membrane of the inner mould element and the first volume of first tissue equivalent;
 - iii. allowing the second tissue equivalent precursor to settle or solidify in the mould cavity of the inner mould element such as to form a tissue implant further comprising a second volume of second tissue equivalent adjacent to the first volume of first tissue equivalent.
10. The process according to claim 8 or 9, wherein the first tissue equivalent is a dermal equivalent comprising fibroblasts suspended in a hydrogel matrix, wherein preferably the hydrogel matrix is a collagen matrix.
 11. The process according to claim 9 or 10, wherein the second tissue equivalent is an epidermal equivalent comprising keratinocytes and preferably is a suspension of keratinocytes in cell culturing medium.
 12. The process according to claim 8 to 11, wherein the tissue implant is a skin implant.

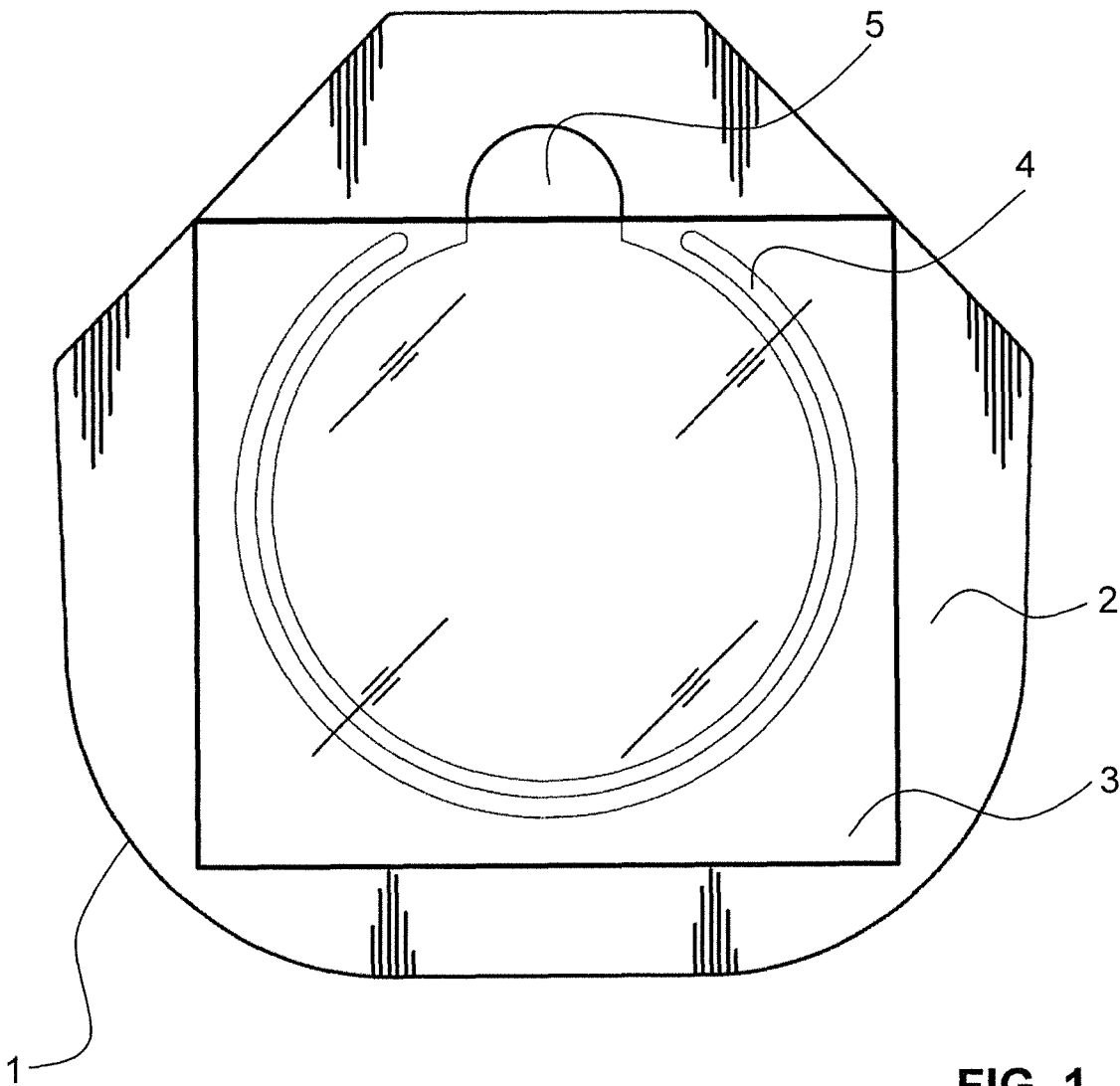


FIG. 1

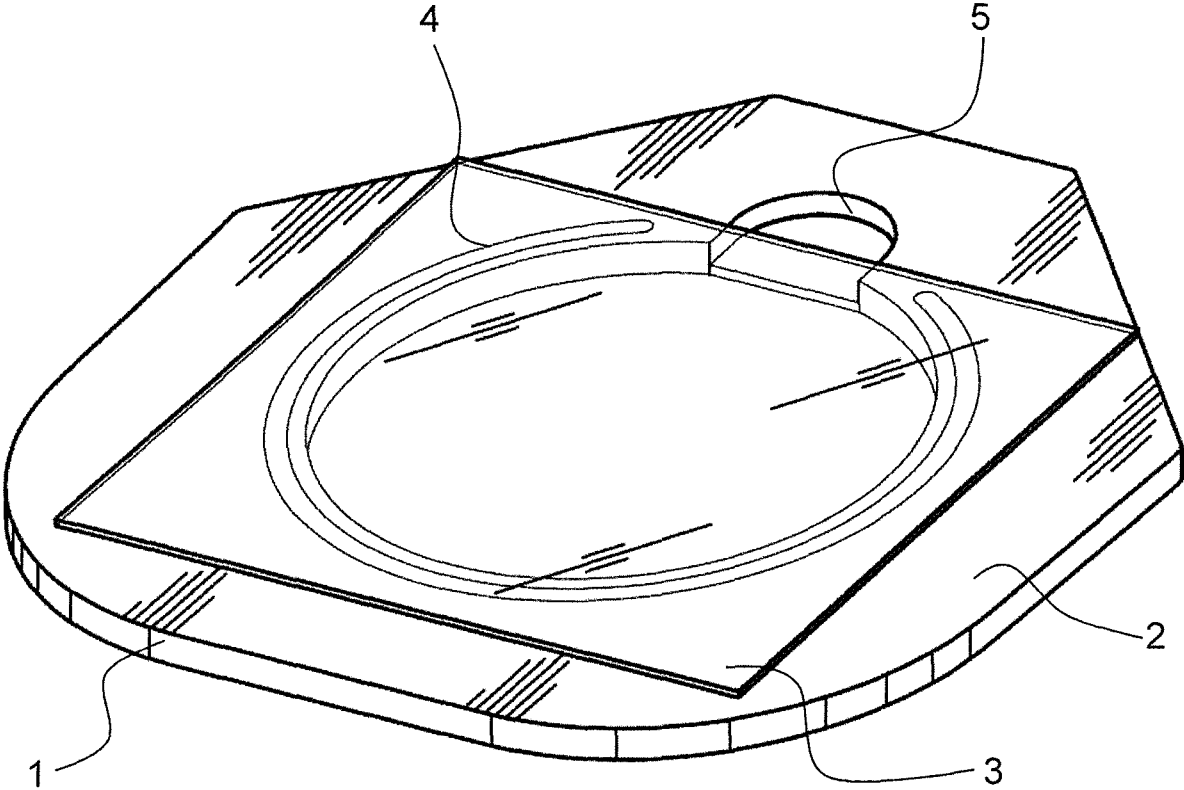
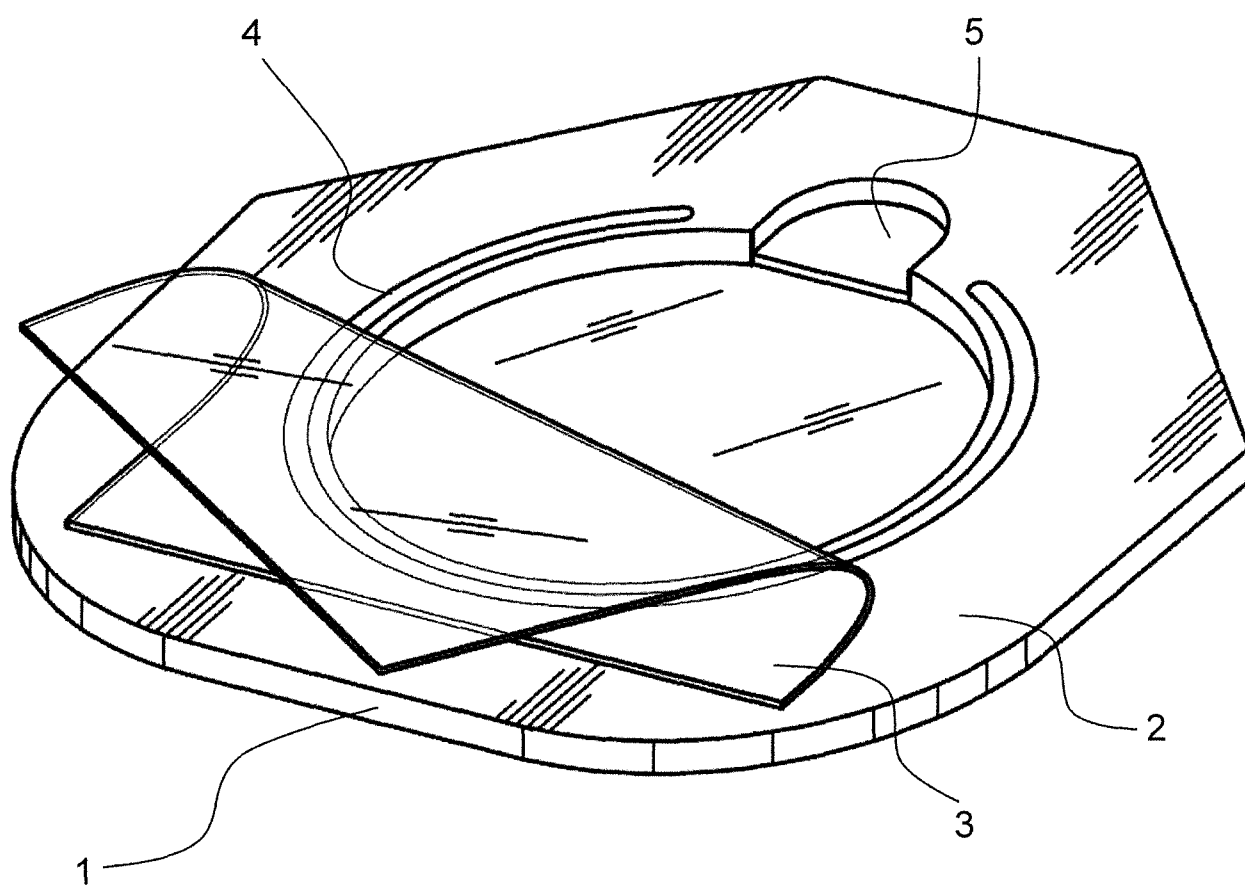


FIG. 2

**FIG. 3**

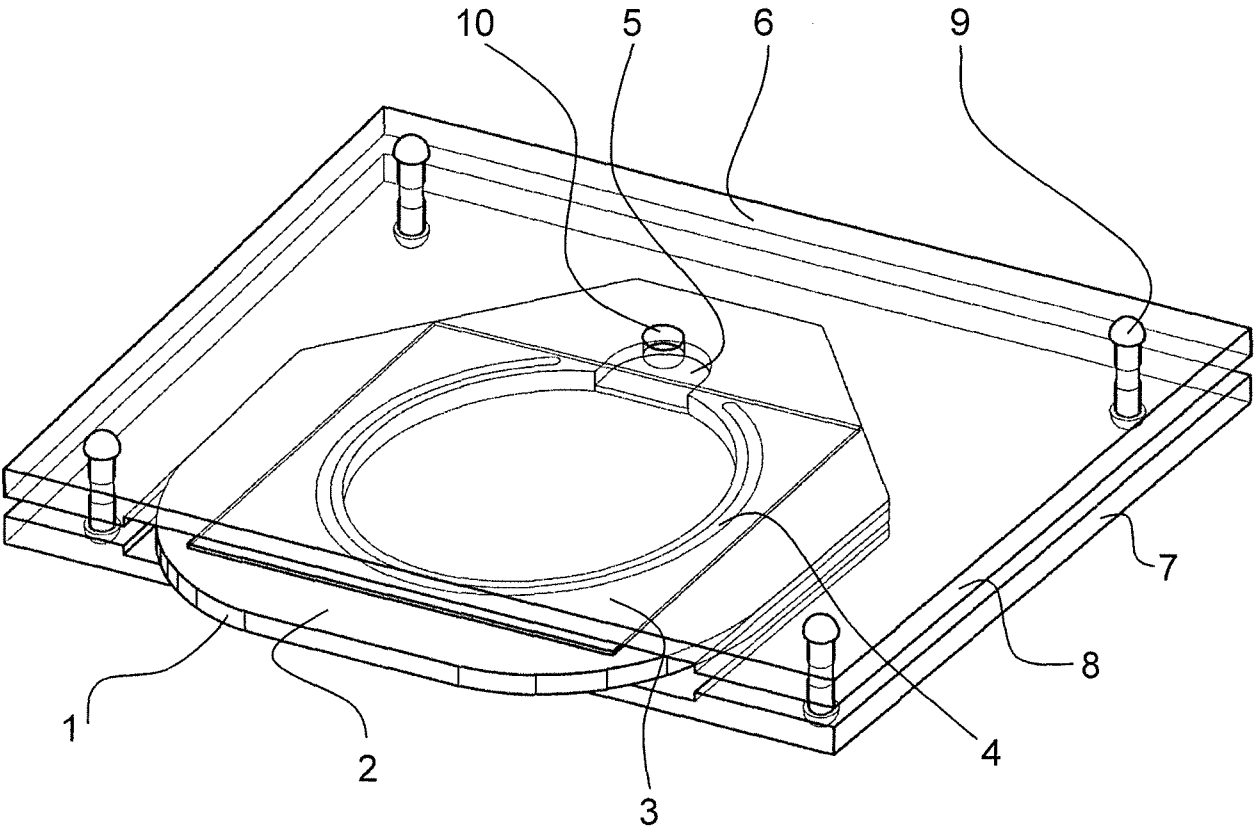
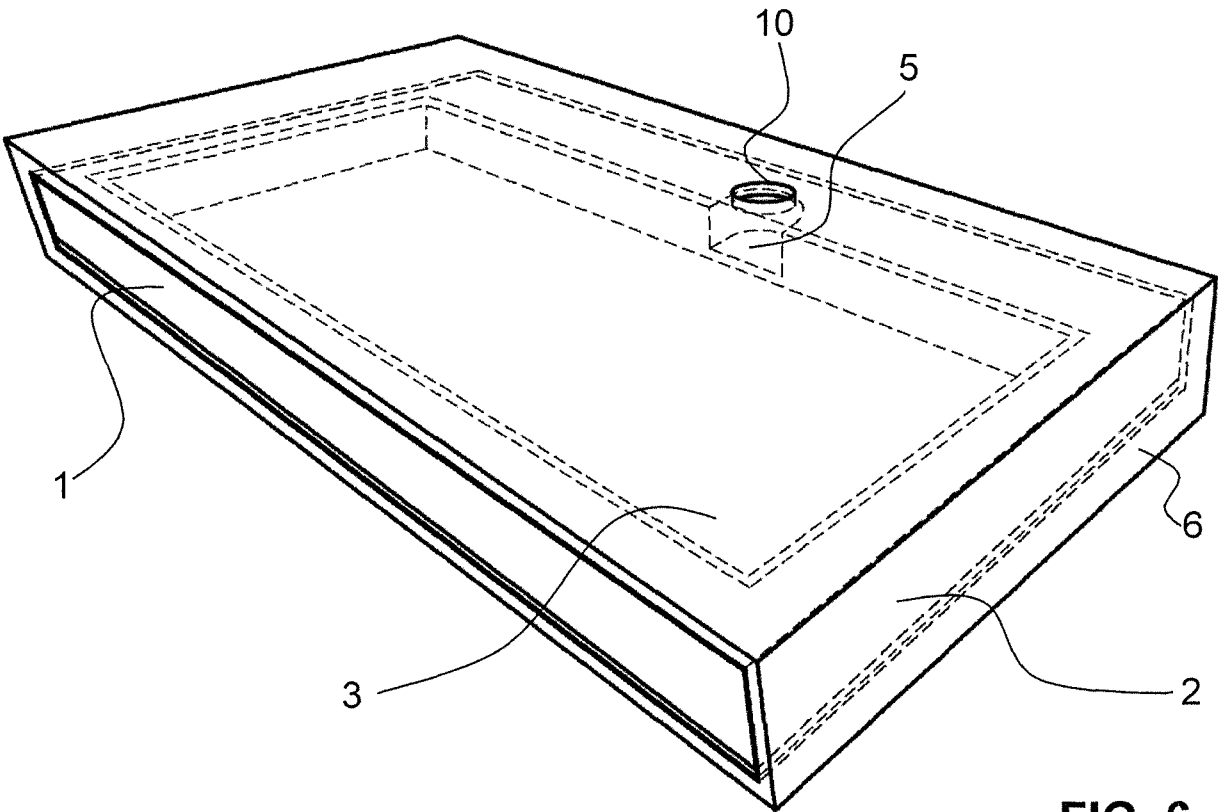
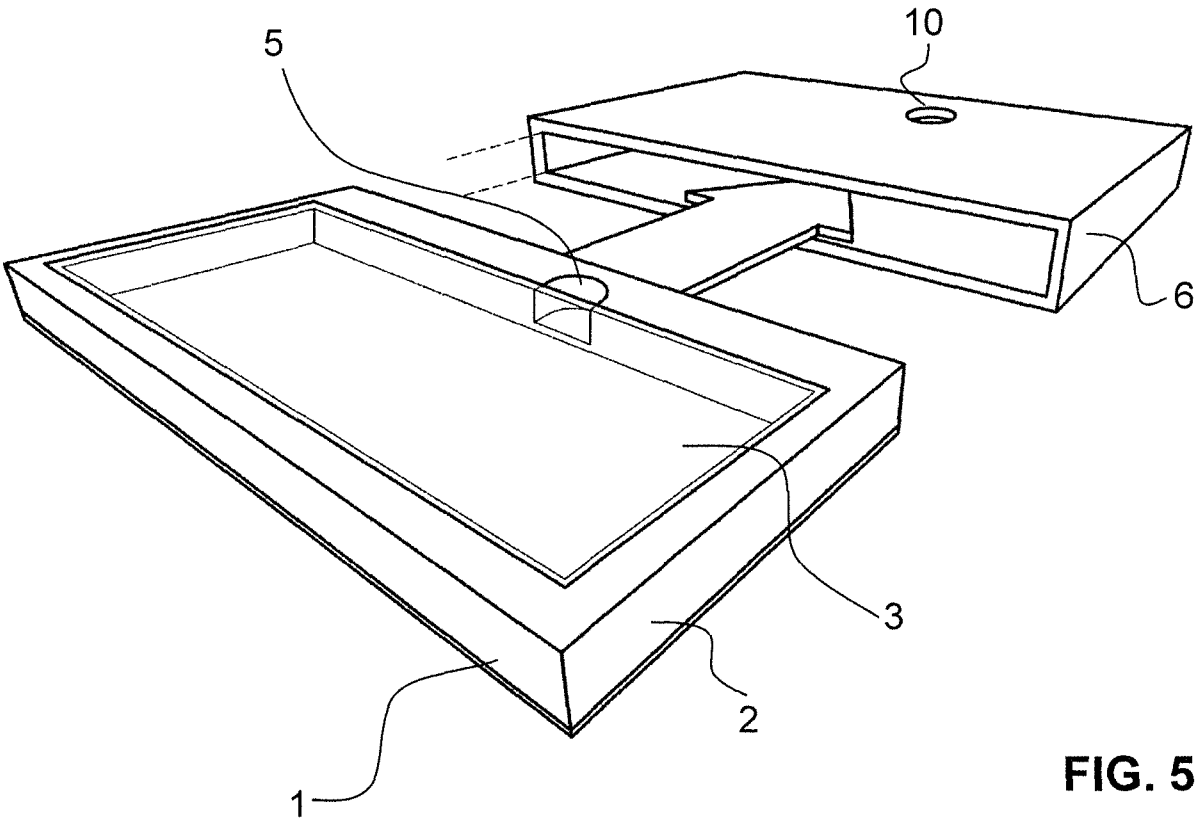


FIG. 4

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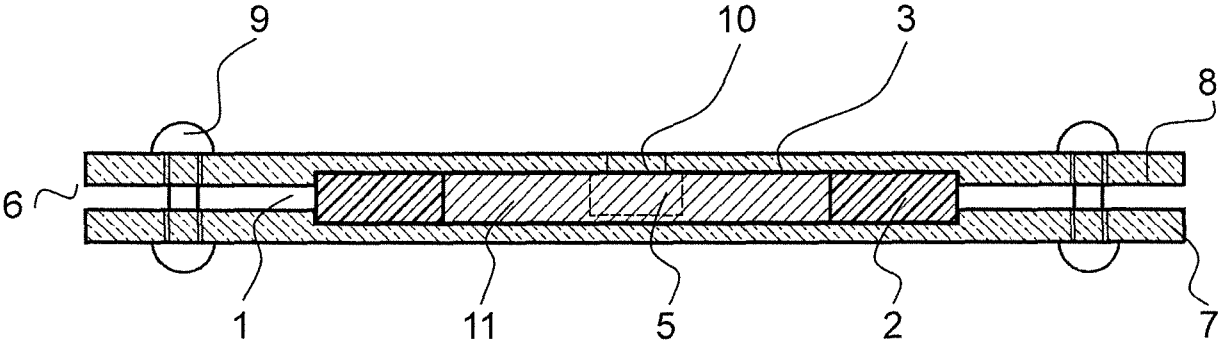


FIG. 7

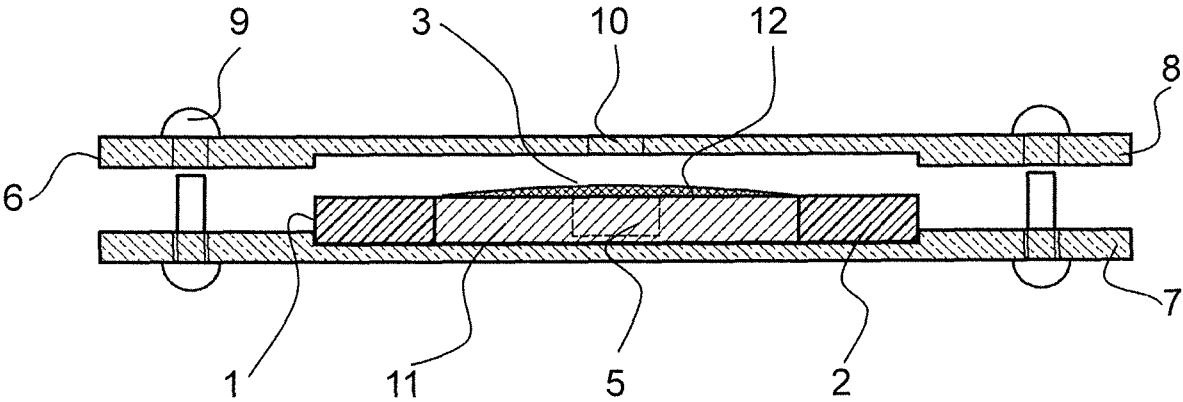


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/064371

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61F2/10 A61L27/60 B29C33/40 B29C33/00 A61L27/38
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61F B29K A61L B29C

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 practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

29 June 2018

Date of mailing of the international search report

10/07/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Alink, Maarten

INTERNATIONAL SEARCH REPORT

International application No
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