



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2018/01/30
(87) Date publication PCT/PCT Publication Date: 2018/08/02
(85) Entrée phase nationale/National Entry: 2019/07/25
(86) N° demande PCT/PCT Application No.: US 2018/015969
(87) N° publication PCT/PCT Publication No.: 2018/140950
(30) Priorité/Priority: 2017/01/30 (US62/451,945)

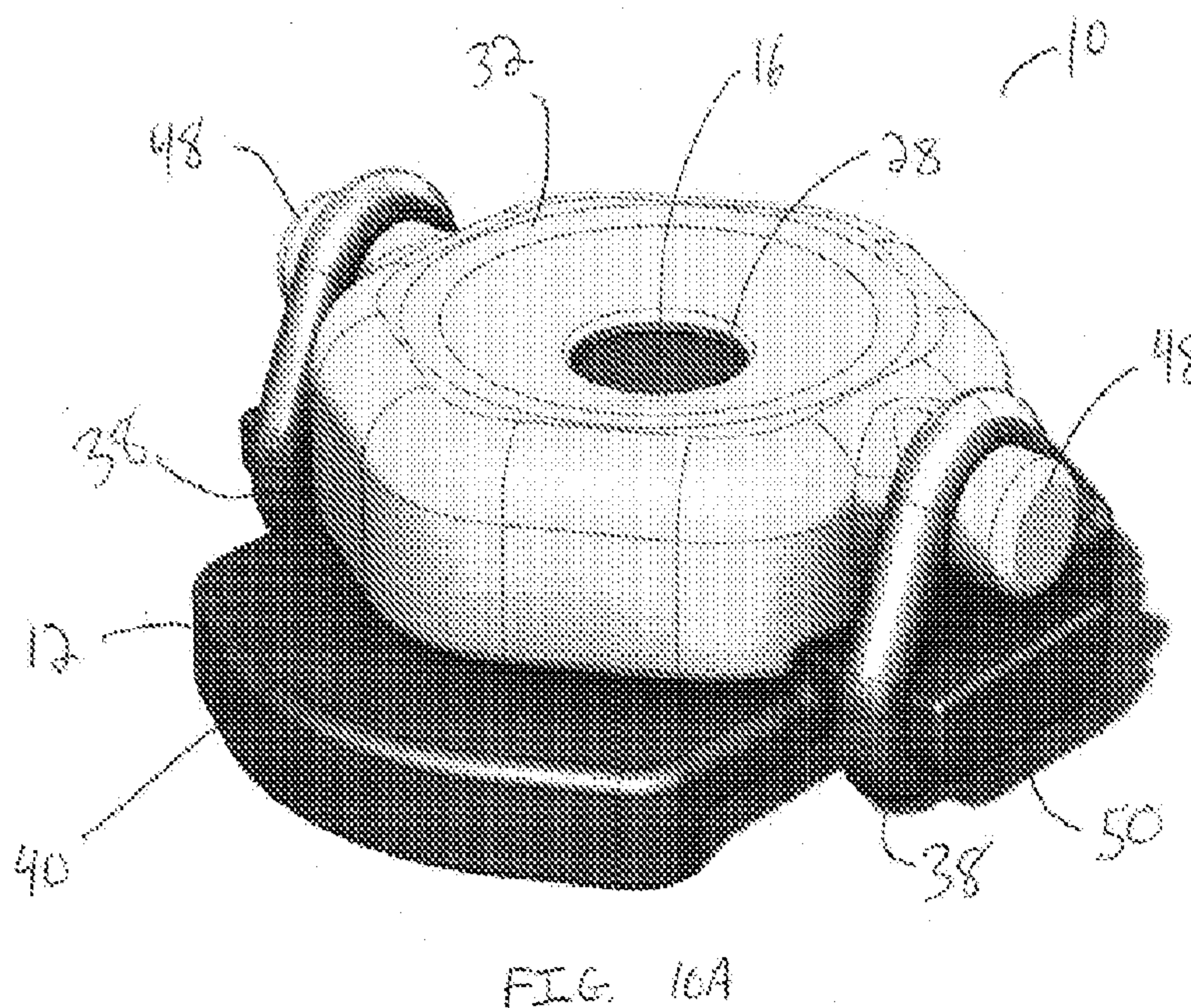
(51) Cl.Int./Int.Cl. *B01D 61/38* (2006.01),
B01D 71/06 (2006.01), *G01N 33/49* (2006.01)

(71) Demandeur/Applicant:
VIVEBIO SCIENTIFIC, LLC, US

(72) Inventeurs/Inventors:
MURRAY, TIMOTHY, US;
BRAUN, DANIEL, US;
WEINBERG, BRIAN, US

(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : DISPOSITIF DE SEPARATION DE PLASMA
(54) Title: PLASMA SEPARATION DEVICE



(57) **Abrégé/Abstract:**

Devices and methods are provided that permit efficient and selective separation of liquid biological specimens into at least two constituent components to facilitate subsequent quantitative and qualitative analysis on at least one analyte of interest in at least one of the components. The devices generally include one or more sample deposition regions supported on a base. Each sample deposition region includes a separation membrane for separating the liquid biological specimen into two different fractions. The first fraction is trapped by the separation membrane while the second fraction passes through the separation membrane and into a respective collection membrane. The separation and collection membranes are easily separable from the devices and can be utilized for further processing and analysis.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
02 August 2018 (02.08.2018)



(10) International Publication Number
WO 2018/140950 A1

(51) International Patent Classification:

B01D 61/38 (2006.01) *G01N 33/49* (2006.01)
B01D 71/06 (2006.01)

(21) International Application Number:

PCT/US2018/015969

(22) International Filing Date:

30 January 2018 (30.01.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/451,945 30 January 2017 (30.01.2017) US

(71) Applicant: **VIVEBIO SCIENTIFIC, LLC** [US/US];
1000 Mansell Exchange West, Suite 350, Alpharetta, Georgia 30022 (US).

(72) Inventors: **MURRAY, Timothy**; 1000 Mansell Exchange West, Suite 350, Alpharetta, Georgia 30022 (US). **BRAUN, Daniel**; 1000 Mansell Exchange West, Suite 350, Alpharet-

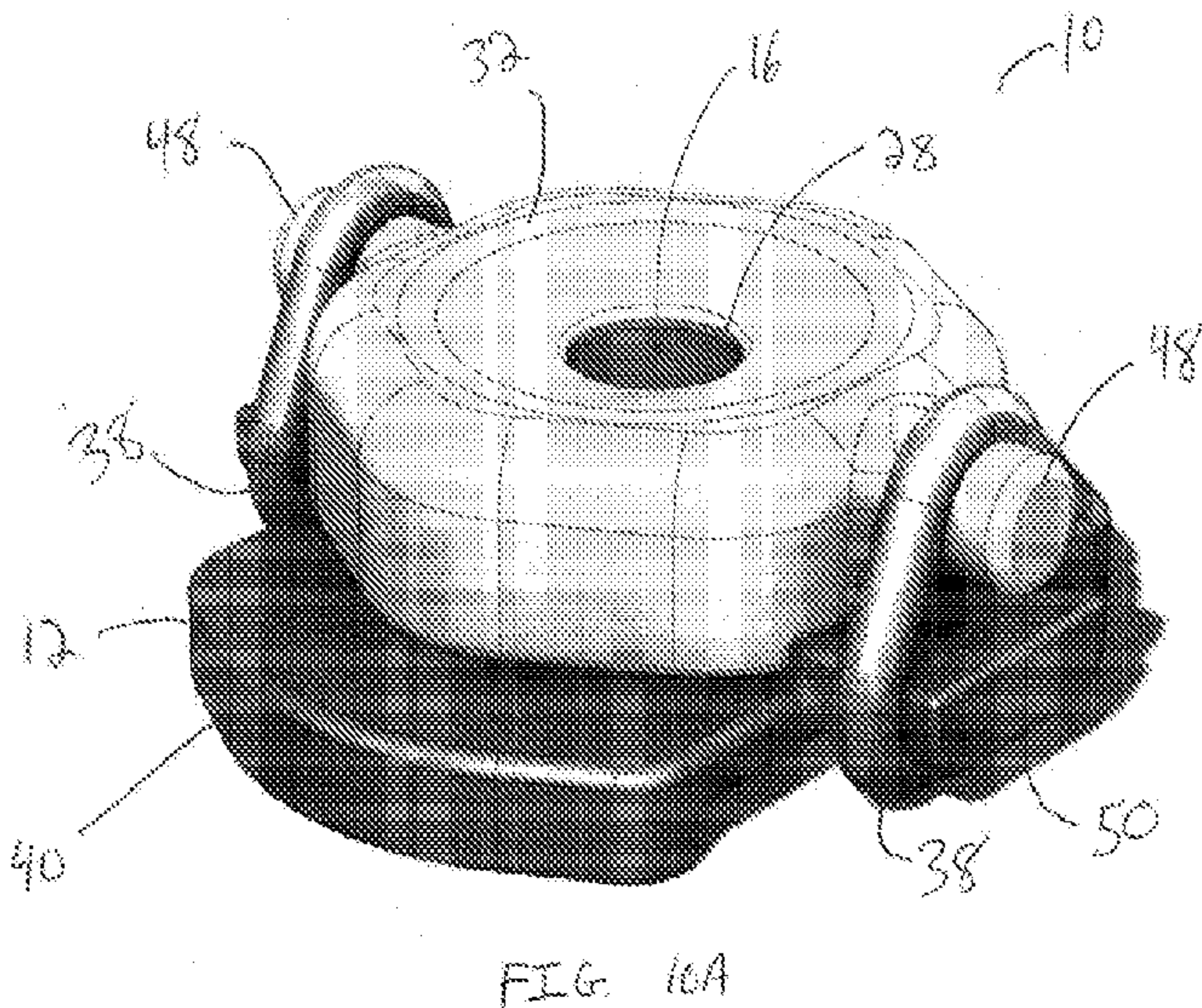
ta, Georgia 30022 (US). **WEINBERG, Brian**; 4305 Sea Bright Drive, Carlsbad, California 92008 (US).

(74) Agent: **WARREN, William L.** et al.; EVERSLEDs SUTHERLAND (US) LLP, 999 PEACHTREE STREET, N.E., SUITE 2300, ATLANTA, Georgia 30309 (US).

(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.

(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,

(54) Title: PLASMA SEPARATION DEVICE



(57) Abstract: Devices and methods are provided that permit efficient and selective separation of liquid biological specimens into at least two constituent components to facilitate subsequent quantitative and qualitative analysis on at least one analyte of interest in at least one of the components. The devices generally include one or more sample deposition regions supported on a base. Each sample deposition region includes a separation membrane for separating the liquid biological specimen into two different fractions. The first fraction is trapped by the separation membrane while the second fraction passes through the separation membrane and into a respective collection membrane. The separation and collection membranes are easily separable from the devices and can be utilized for further processing and analysis.

WO 2018/140950 A1

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))*

-1-

PLASMA SEPARATION DEVICE

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/451,945, filed on January 30, 2017, the disclosure of which is incorporated herein
5 by reference in its entirety.

FIELD OF INVENTION

[0002] The present disclosure generally relates to devices and methods that permit efficient and selective separation of liquid biological specimens (e.g. biological fluids or biological specimen containing suspensions) into at least two constituent components to
10 facilitate subsequent quantitative and qualitative analysis on at least one analyte of interest in at least one of the components.

BACKGROUND OF THE INVENTION

[0003] Biological specimens are often collected for analysis of the levels and concentrations of various analytes contained therein. Although many diagnostics are
15 carried out on biological specimens in their native state, many times the biological specimen must be separated into its constituent components for a variety of reasons. Separating a biological specimen into different constituent parts can maximize the precision, accuracy, and reproducibility of detecting and quantifying analytes of interest within the biological specimen. For example, it is often necessary to filter out solid
20 components from whole blood (e.g., white blood cells, red blood cells, etc.), separate blood serum from whole blood, and separate blood plasma from whole blood, to improve not only the recovery of select analytes from the biological specimen (e.g. viruses, plasma proteins, cytokines, chemokines, immunoglobins, etc.) but also improve the subsequent detection and analysis of those analytes. As one example, red blood cells (erythrocytes)
25 scatter and absorb light and, therefore, can adversely affect diagnostic tests that rely on measurements of either reflected or transmitted light. Removing red blood cells can help obtain the most accurate reading possible.

[0004] Traditionally, liquid biological specimens have been separated by centrifugation. For example, blood plasma and serum have been separated from whole
30 blood by centrifuging either before (for plasma) or after (for serum) clotting. However,

-2-

centrifugation requires electricity and expensive equipment that may not be readily available in a clinical laboratory or out in the field. Further, centrifugation can damage analytes of interest (e.g. nucleic acids such as DNA and RNA).

[0005] A number of techniques have been devised to avoid this problem. The techniques generally utilize a filtering device that separates a liquid biological specimen into various components. However, these devices have proven to be unsuitable for a variety of reasons. Therefore, what are needed are improved devices and methods that permit efficient and selective separation of liquid biological specimens into at least two constituent components to facilitate subsequent quantitative and qualitative analysis on at least one analyte of interest in at least one of the components.

SUMMARY OF THE INVENTION

[0006] This disclosure generally provides liquid biological specimen separation devices and methods of using the same. In some aspects, a liquid biological specimen separation device is provided that comprises a base; a collection membrane disposed on the base, a separation membrane disposed on the collection membrane; and a cover disposed on the separation membrane, wherein the cover comprises an aperture therein configured to allow deposition of a liquid biological specimen. In other aspects, a liquid biological specimen separation device is provided that comprises a base; a plurality of collection membranes disposed on the base; a plurality of separation membranes, wherein each separation membrane is disposed on a corresponding one of the collection membranes; and a plurality of covers, wherein each cover is disposed on a corresponding one of the separation membranes, and wherein each cover comprises an aperture therein configured to allow deposition of a liquid biological specimen therethrough. In other aspects, a liquid biological specimen separation device is provided that comprises a base; a collection membrane disposed in the base, a separation membrane disposed in the base and on the collection membrane, and a cap disposed on the separation membrane and base, wherein the cap comprises an aperture therein configured to allow deposition of a liquid biological specimen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Referring now to the drawings, which are meant to be exemplary and not limiting, and wherein like elements are numbered alike. The detailed description is set

-3-

forth with reference to the accompanying drawings illustrating examples of the disclosure, in which use of the same reference numerals indicates similar or identical items. Certain embodiments of the present disclosure may include elements, components, and/or configurations other than those illustrated in the drawings, and some of the elements, components, and/or configurations illustrated in the drawings may not be present in certain
5 embodiments.

[0008] **FIGS. 1A-1B** show a liquid specimen separation device in accordance with one example embodiment of the disclosure.

[0009] **FIG. 2** shows an exploded view of a liquid specimen separation device in
10 accordance with one example embodiment of the disclosure.

[0010] **FIGS. 3A-3B** show a liquid specimen separation device in accordance with a second example embodiment of the disclosure.

[0011] **FIGS. 4A-4B** show a liquid specimen separation device in accordance with a third example embodiment of the disclosure.

15 [0012] **FIG. 5** shows an exploded view of a liquid specimen separation device in accordance with a third example embodiment of the disclosure.

[0013] **FIG. 6** shows an exploded view of a liquid specimen separation device in accordance with a fourth example embodiment of the disclosure.

[0014] **FIG. 7** shows cut away view of a liquid specimen separation device in
20 accordance with a fourth example embodiment of the disclosure.

[0015] **FIG. 8** shows a liquid specimen separation device in accordance with a fourth example embodiment of the disclosure.

[0016] **FIGS. 9A-9D** show a liquid specimen separation device in accordance with a fifth example embodiment of the disclosure.

25 [0017] **FIGS. 10A-10B** show a liquid specimen separation device in accordance with a sixth example embodiment of the disclosure.

[0018] **FIGS. 11A-11F** show a liquid specimen separation device in accordance with a seventh example embodiment of the disclosure.

DETAILED DESCRIPTION OF THE INVENTION

[0019] Devices and methods are provided that permit efficient and selective separation of liquid biological specimens into at least two constituent components to facilitate subsequent quantitative and qualitative analysis on at least one analyte of interest in at least one of the components. The devices and methods fulfill the need for a convenient and simple method for filtering, separating, and/or storing an analyte of interest.

[0020] Examples of biological specimen suitable for use with devices described herein include whole blood, plasma, urine, saliva, sputum, semen, vaginal lavage, bone marrow, breast milk, and cerebrospinal fluid. One advantage of the present devices is that they can sufficiently preserve analytes of interest.

[0021] The devices generally include one or more sample deposition regions supported on an ergonomically configured base that permits ease of handling. Each sample deposition region includes a separation membrane for separating the liquid biological specimen into two different fractions. The first fraction is trapped by the separation membrane while the second fraction passes through the separation membrane and into a respective collection membrane. The separation and collection membranes are easily separable from the devices and can be utilized for further processing and analysis. The devices can include other features such as one or more covers (e.g. stickers) or caps that define the sample deposition regions, secure the separation membranes, collection membranes, and/or base together or in place, urge the separation membranes, collection membranes, and/or base together, and allows for easy removal of the separation and/or collection membranes from the device. The devices can also include identification markers (e.g. barcodes) on the base.

[0022] The devices can be used to trap and filter out solid components from a liquid biological specimen. For example, the devices can include a separation membrane that filters and traps solid components of a whole blood specimen (e.g. red blood cells, white blood cells, erythrocytes), thereby resulting in the collection membrane absorbing cell-free serum, plasma, and plasma proteins.

[0023] As used herein, the term “analyte” refers to any micro- or macro- molecules in a biological specimen that are to be detected or analyzed. These include, for example,

-5-

nucleic acids (e.g. DNA, RNA), polynucleotides, oligonucleotides, proteins, polypeptides, oligopeptides, enzymes, amino acids, receptors, carbohydrates, lipids, whole cells, cellular fragments, any intra- or extra- cellular molecules and fragments, viruses, viral molecules and fragments, bacteria, and the like. In certain embodiments, the analytes are exogenous
 5 natural or synthetic compounds such as small molecules like drugs, prodrugs, and metabolites thereof. In certain embodiments, the analytes are nucleic acids such as proviral and/or viral DNA and/or RNA such as, for example, proviral and/or viral nucleic acids from human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), influenza, parvovirus B19, or any other human or animal viral pathogen. In
 10 certain embodiments, the analytes are viral particles for determining viral load. In certain embodiments, the analytes are biological markers for determining HLA blood types, useful for molecular diagnostic genotyping. In certain embodiments, the analytes are inflammatory biomarkers such as CXCL9/MIG and CXCL10/IP-10. In certain embodiments, the analytes are micronutrients such as Folic Acid, Homocysteine, Retinol
 15 Binding Protein (and/or Vitamin A), Thyroglobulin, Vitamin D, trace metals (e.g. zinc), Ferritin, Transferrin Receptors, Methylmalonic Acid, Holo-Transcobalamin, C-Reactive Protein, and alpha-Acid Glycoprotein.

[0024] “Biological specimen” refers to biologic samples, either in liquid or solid form, having contained therein an analyte of interest. A biological specimen can be, for
 20 example, whole blood, plasma, serum, lymph, synovial fluid, bone marrow, cerebrospinal cord fluid, semen, saliva, urine, feces, sputum, vaginal lavage, skin scrapings, hair root cells, or the like of humans or animals; physiological and pathological body liquids such as secretions, excretions, exudates and transudates; any cells or cell components of humans, animals, plants, bacteria, fungi, plasmids, viruses, parasites, or the like that
 25 contain analytes of interest, and any combination thereof. In certain embodiments, a biological specimen can be a human body fluid such as whole blood, which can contain analytes of interest such as proviral nucleic acids and/or plasma proteins such as Troponin, monoclonal kappa and lambda free light chains, Cystatin C, and Carbohydrate-Deficient Transferrin (CDT).

30 **[0025]** “Liquid biological specimen” means a biological fluid or a biological specimen suspended in a fluid medium (e.g. water, saline, etc.). Exemplary liquid biological specimens include human, animal, plant, bacteria, fungi, plasmids, viruses,

-6-

parasites (e.g. helminthes, protozoas, spirochetes) extracts or suspensions; liquid extracts or homogenates of human or animal body tissues (e.g., bone, liver, kidney, brain); media from DNA or RNA synthesis; mixtures of chemically or biochemically synthesized DNA or RNA; and body fluids/liquids such as whole blood, plasma, serum, synovial fluid, cerebrospinal cord fluid, semen, and saliva.

I. DEVICES

[0026] Embodiments of liquid biological specimen separation devices provided herein generally comprise a base support, one or more collection membranes disposed on the base, one or more separation membranes disposed on the collection membranes, and one or more covers disposed on the one or more separation membranes, as illustrated by the non-limiting embodiments shown in **FIGS. 1A-11F**. One embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 1A-2**. Another embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 3A-3B**. Another embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 4A-5**. Another embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 6-8**. Another embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 9A-9D**. Another embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 10A-10B**. Another embodiment of a liquid biological specimen separation device is illustrated in **FIGS. 11A-11F**.

[0027] **FIGS 1A-11F** show a liquid biological specimen separation device **10**. The separation device **10** can include base **12**, collection membrane(s) **14**, separation membrane(s) **16**, cover(s) **18**, collection membrane support(s) **20**, and/or cap(s) **32**.

[0028] Base **12** generally functions as a supporting surface. Base **12** can also function to secure the base **12**, collection membranes **14**, separation membranes **16**, covers **18**, and/or collection membrane supports **20** together. Base **12** can also serve as a protective enclosure that protects any components that may be contained therein (e.g. collection membranes **14**, separation membranes **16**, covers **18**, collection membrane supports **20**, biological specimens, and/or analytes enclosed by base **12**) from outside influences or effects.

[0029] Base **12** can assume any dimensions, size, and shape suitable for serving as a support in a liquid biological specimen separation device **10**. For example, the general

-7-

shape of base **12** can be round, rectangular, oval, square, trapezoidal, triangular, pentagonal, hexagonal, octagonal, ellipsoid, crescent, curvilinear, egg, quatrefoil, cinquefoil, and the like. Base **12** can have a uniform shape. Base **12** can have a shape that includes one or more lobes/projections extending therefrom. Base **12** can have surfaces
 5 that are uniformly flat. Base **12** can be entirely flat. Base **12** can have a three dimensional, freeform structure. Base **12** can have one or more recesses dimensioned to receive, or projections dimensioned to extend into, and complementary fit collection membrane(s) **14**, separation membrane(s) **16**, cover(s) **18**, collection membrane support(s) **20**, and/or caps **32**. Base **12** can have a projection extending therefrom shaped and
 10 dimensioned to receive or contain collection membrane(s) **14**, separation membrane(s) **16**, cover(s) **18**, collection membrane support(s) **20**, and/or caps **32**.

[0030] Base **12** can have complementary features that permit separation device **10** to have an open configuration and a closed configuration. For example, base **12** can have a well portion and a cap portion that assume a first position wherein the well portion and
 15 the cap portion extend away from one another (open configuration) and a second position wherein the well portion and the cap portion complementary mate with one another (closed configuration). As another example, base **12** can have a raised inner projection and a cap that assume a first position wherein the raised inner projection and the cap are separated from one another (open configuration) and a second position wherein the raised
 20 inner projection and the cap complementary mate with one another (closed configuration).

[0031] Base **12** can have one or more sample deposition apertures **28** therethrough that define an area for depositing a liquid biological specimen. Base **12** can have features that improve handling and/or use of separation device **10**. For example, base **12** can include features, projections, tabs, handles, and the like that facilitate gripping,
 25 holding, and manipulating separation device **10** (e.g. a tab for use in opening separation device **10** when in a closed configuration).

[0032] Separation device **10** can have any suitable number of biological specimen receiving/separation/collection regions such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more such regions.

30 **[0033]** Base **12** can be made of any suitable material, preferably one that provides sufficient flexibility/stiffness and strength. Base **12** can be made of, for example, suitable

-8-

plastics materials (e.g. polyethylene, acrylic, polypropylene), paper materials (e.g. cardstock, cardboard, etc.), and the like.

[0034] Base **12** can include an identifier **22** thereon or therein. The identifier **22** can be integrated/disposed into or onto an outer surface of base **12**. For example, identifier **22** can be printed on or attached (e.g. adhesive label) to base **12**. The identifier **22** can also be integrated/disposed in base **12**. The identifier **22** can use any suitable identification technology. For example, identifier **22** can be any suitable RFID chip, single-dimensional (1D) barcodes, two-dimensional (2D) barcodes, QR codes, alpha-numeric codes, and the like.

10 [0035] Identifier **22** can store or have associated therewith identification information. Identification information can include information specific to a patient associated with a biological specimen stored therein, including personal information (address, name, sex, date of birth, ethnic background, etc.) and/or biometric information (e.g., a fingerprint, a facial image or template). Identifier **22** can also store or have
15 associated therewith contextual information such as time, date, location of testing, and the like. To protect identifier **22** and any information associated therewith, a layer of overlamine or other protective material may additionally be provided over identifier **22**.

[0036] One or more collection membranes **14** can be disposed on base **12**. One or more collection membrane supports **20** can also be disposed on base **12**. A collection
20 membrane **14** can be disposed in a collection membrane support **20**. The collection membranes **14** generally function to absorb the fraction of a liquid biological specimen that flows through separation membrane **16**. The collection membrane supports **20** generally function to support (e.g. hold in place) the collection membranes **14**.

[0037] Collection membranes **14** can be reversibly adhered to or held in place on
25 base **12** by any suitable means. For example, collection membrane **14** can be secured in place by cover **18** (e.g. a sticker), the sticker being adhered to base **12** and having sandwiched there between collection membrane **14**. As another example, collection membrane **14** can be secured in place by an interference fit with another feature of separation device **10** (e.g. the collection membrane **14** is disposed in, and has an
30 interference fit with, a well in base **12**). As another example, collection membrane **14** can be secured in place by disposing collection membrane **14** in a recess of base **12**

-9-

dimensioned to fit and receive collection membrane **14**. As another example, collection membrane **14** can be secured in place by securing collection membrane **14** in between a well portion and a cap portion of base **12** that complementary mate with one another (a closed configuration). The collection membrane **14** can be secured in between the well
5 portion and the cap portion of base **12** along with other features (e.g. separation membrane **16**), and the stacked features can be collectively dimensioned to prevent or reduce movement of the features within or out of separation device **10**. As another example, collection membrane **14** can be secured in place by securing collection membrane **14** in between a raised inner projection of base **12** and a cap **32** that complementary mate with
10 one another (a closed configuration). The collection membrane **14** can be secured in between a raised inner projection of base **12** and a cap **32** along with other features (e.g. separation membrane **16**), and the stacked features can be collectively dimensioned to prevent or reduce movement of the features within or out of separation device **10**.

[0038] As another example, in embodiments that include collection membrane supports **20**, collection membranes **12** can be connected or attached to collection membrane supports **20**, the collection membrane supports **20** being adhered to, fixedly attached to, or held in place on, base **12** by any suitable means (e.g. adhesives, interference fit into a well of the base). A benefit of this approach is that collection membranes **14** can be easily separated from both base **12** and collection membrane supports **20**. For example,
20 the interface between a collection membrane **14** and a collection membrane support **20** can include a series of perforations that enable easy removal of collection membrane **14** from collection membrane support **20**. This facilitates further processing of the biological specimen and/or analytes of interest absorbed by collection membrane **14**.

[0039] In embodiments having both collection membrane(s) **14** and collection membrane support(s) **20**, the collection membranes **14** and collection membrane supports **20** can be a unitary item (e.g. a single piece of whatman 903 paper having a sample collection region and a supporting region) or discrete items from one another (e.g. a first piece of whatman 903 paper is a collection membrane and a second piece of whatman 903 paper is a collection membrane support).

30 [0040] The collection membrane(s) **14** and collection membrane support(s) **20** can be made of the same material or different materials. The collection membranes **14** and collection membrane supports **20** can be made of any suitable material. Suitable materials

-10-

include, for example, plastics, polymers, cotton, cellulose, and/or paper. In some embodiments, the collection membrane(s) **14** and/or collection membrane support(s) **20** are filter papers. Filter papers that may be selected for use include cellulose fiber papers manufactured from cotton linters. Cotton linters (i.e., cotton wool) are short fibers that
5 adhere to seeds of a cotton plant after the longer fibers have been pulled from the cotton seed. Filter papers can also include filter papers for blood collection registered by the U.S. Food and Drug Administration as Class II Medical Devices (21 CFR §862.1675), such as WHATMAN 903, AHLSTROM 142, AHLSTROM 226, AHLSTROM 222, AHLSTROM 238, AHLSTROM 270, ALHSTROM 601, and ESSENTRA. In some embodiments, a
10 majority of the cellulose fibers of a cellulose fiber filter paper may have sizes in the range of about 1-100 microns, 10-50 microns, or 20-25 microns in length and may contain numerous hydrophobic and/or hydrophilic pockets.

[0041] Collection membranes **14** and collection membrane supports **20** can have any suitable shape such as, for example, a circle, oval, square, rectangle, triangle,
15 hexagonal, or other shapes and surface textures suitable for use in the devices described herein. Collection membrane support **20** can have a collection membrane aperture **24** for receiving collection membranes **14**. Collection membranes **14** and collection membrane supports **20** can also be dimensioned in a manner that facilitates removing collection membranes **14** from collection membrane supports **20**. For example, collection membrane
20 supports **20** can include removal apertures **26** that allow a device (e.g. tongs) to selectively pincer and remove collection membranes **14** from collection membrane supports **20**.

[0042] Separation device **10** can include one or more collection membranes **14** and/or one or more collection membrane supports **20**. For example, separation device **10** can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more
25 collection membranes **14** and/or collection membrane supports **20**. The number of collection membranes **14** and the number of collection membrane supports **20** in separation device **10** can be the same or they can be different. For example, in some embodiments separation device **10** can have four collection membranes **14** and four collection membrane supports **20** while in other embodiments separation device **10** can
30 have four collection membranes **14** and one collection membrane support **20**.

[0043] Collection membranes **14** and/or collection membrane supports **20** can have any suitable size. In certain embodiments, collection membranes **14** and/or collection

-11-

membrane supports **20** can have a diameter/width of from about 1mm to 50 mm, or from 10 mm to 30 mm, inclusive. In some embodiments, collection membranes **14** have a diameter/width of from about 1 mm to about 15 mm.

[0044] Collection membranes **14** can include a composition absorbed to a surface thereof, wherein the composition protects against degradation of an analyte of interest disposed therein. Protection against degradation may include protection against substantial damaging of analytes of interest caused by chemical or biological agents including action of bacteria, free radicals, nucleases, ultraviolet radiation, oxidizing agent, alkylating agents, or acidic agents (e.g., pollutants in the atmosphere). In certain embodiments, the composition absorbed on the collection membrane **14** can include one or more of a weak base, a chelating agent, a protein denaturing agent such as a detergent or surfactant, a nuclease inhibitor, a free radical trap, and an oxygen scavenger element. As used herein, a “weak base” can be a Lewis base which has a pH of about 6 to 10, preferably about pH 8 to 9.5. In a case where the stored analyte of interest is RNA, particularly unstable RNA, the composition may include RNase inhibitors and inactivators, genetic probes, complementary DNA or RNA (or functionally equivalent compounds), proteins and organic moieties that stabilize RNA or prevent its degradation.

[0045] One or more separation membranes **16** can be disposed on base **12**, collection membranes **14**, covers **18** and/or collection membrane supports **20**. The separation membranes **16** generally function to separate a liquid biological specimen into a first fraction and a second fraction. The first fraction is contained/trapped within separation membranes **16** while the second fraction passes through separation membranes **16** and into collection membranes **14**.

[0046] Separation membranes **16** can be reversibly held in place on base **12**, collection membranes **14**, covers **18**, and/or collection membrane supports **20** by any suitable means. For example, separation membranes **16** can be secured in place by cover **18** (e.g. a sticker), the sticker being adhered to base **12** and/or collection membrane supports **20** and having sandwiched there between collection membranes **14**, collection membrane supports **20**, and/or separation membranes **16**. The compressive force keeps separation membranes **16** in contact with collection membranes **14**. In another example, separation membranes **16** can be secured in place by cover **18** (e.g. a sticker), the sticker being adhered to base **12** and having sandwiched there between separation membranes **16**.

-12-

In another example, separation membranes **16** can be secured in place by an interference fit with another feature of separation device **10** (e.g. the separation membrane **16** is disposed in, and has an interference fit with, a well in base **12**). As another example, separation membrane **16** can be secured in place by disposing separation membrane **16** in a recess of base **12** dimensioned to fit and receive separation membrane **16**.

[0047] As another example, separation membrane **16** can be secured in place by securing separation membrane **16** in between a well portion and a cap portion of base **12** that complementary mate with one another (a closed configuration). The separation membrane **16** can be secured in between the well portion and the cap portion of base **12** along with other features (e.g. collection membrane **14**), and the stacked features can be collectively dimensioned to prevent or reduce movement of the features within or out of separation device **10**. As another example, separation membrane **16** can be secured in place by securing separation membrane **16** in between a raised inner projection of base **12** and a cap **32** that complementary mate with one another (a closed configuration). The separation membrane **16** can be secured in between a raised inner projection of base **12** and a cap **32** along with other features (e.g. collection membrane **14**), and the stacked features can be collectively dimensioned to prevent or reduce movement of the features within or out of separation device **10**.

[0048] Both collection membranes **14** and separation membranes **16** are easily separable from separation device **10** so that collection membranes **14** and separation membranes **16** can be accessed and removed from separation device **10** for further analysis.

[0049] Separation membranes **16** are generally made of a material that allows for flow of a liquid biological specimen or a fraction thereof therethrough. Separation membranes **16** can comprise a plurality of fibers. Separation membranes **16** can be made of a material that has a gradually decreasing pore size (e.g. an asymmetric porous membrane) from a top side (e.g. the side where biological specimens are initially deposited) to a bottom side (the side in contact with collection membrane **14**). Separation membrane **16** can also be made of a material that has a uniform pore size throughout. In certain embodiments, flow of a liquid biological specimen deposited on separation membrane **16** through separation membrane **16** is driven by capillary forces (e.g. capillary flow) and/or gravity. In certain embodiments, materials suitable for use in separation

-13-

membranes **16** are those in which one biological specimen moves faster through the separation membrane than another biological specimen (e.g. blood plasma moves faster than corpuscles).

[0050] Suitable materials for use in separation membranes **16** can include, for example, synthetic polymers having fine fiber diameter and fibers made of glass or porous polymers. In certain preferred embodiments, separation membranes **16** are made of a polysulfone polymer material having a porosity that gradually decreases from a top side of the membrane to a bottom side of the membrane so as to filter and trap solid and/or liquid components of a liquid biological specimen deposited on separation membranes **16**. Separation membrane materials can include, for example, synthetic or natural polymers such as cellulose mixed esters, polyvinylidene difluoride, polytetrafluoroethylene, polycarbonate, polypropylene, polyester, and polysulfone polymers and matrices (e.g., asymmetric sub-micron polysulfone (BTS) and/or asymmetric super micron polysulfone (MMM) made by Pall Corporation). Separation membrane materials can also include, for example, VIVID GR, VIVID GX, and CYTOSEP 1660. A person of ordinary skill will readily appreciate that other membranes or filtering materials can be used. In some embodiments, separation membranes **16** are suitable for blood component filtering and serum/plasma separation. In some embodiments, separation membranes **16** have a porosity of not more than 30%, and preferably not more than 25%. In certain embodiments, separation membranes **16** can be made of polysulfone polymer having a pore size ranging from about 0.1-20 microns and a pore size ratio from about 50:1 to 100:1.

[0051] The size of a separation membrane **16** can be larger than the size of a collection membrane **14** to which it is removably disposed upon. For example, the size of a separation membrane **16** may be at least 20%, or at least 30%, or at least 40%, or at least 50% larger than a size of a corresponding collection membrane **14**. Alternatively, the size of a separation membrane **16** can be the same or about the same (e.g. within 10% by area) size as a collection membrane **14** to which it is removably disposed upon. Alternatively, the size of a separation membrane **16** can be smaller than the size of a collection membrane **14** to which it is removably disposed upon. In certain embodiments, a separation membrane **16** has a diameter/width of from about 1mm to 50 mm, or from 10

-14-

mm to 30 mm, inclusive. For example, separation membrane **16** can have a diameter/width of about 10 mm to about 20 mm.

[0052] Separation membrane **16** can have a shape that is the same shape as a corresponding collection membrane **14** (e.g. circles). Separation membrane **16** can also have a shape that is different from a shape of a corresponding collection membrane **14** and, thus, does not align in its entirety with the shape of collection membrane **14** when brought into contact thereto. For example, separation membrane **16** can have an irregular or oblong shape (e.g., a racquet shape with a handle-like extension extending on a lateral side thereof) whereas collection membrane **14** can have a circular shape.

[0053] Separation device **10** can include one or more separation membranes **16**. For example, separation device **10** can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more separation membranes **16**.

[0054] One or more covers **18** can be disposed on separation membranes **16**, collection membranes **14**, collection membrane supports **20**, and/or base **12**. The covers **18** generally have one or more sample deposition apertures **28** therethrough that define an area for depositing a liquid biological specimen. The covers **18** can also function to secure the separation membranes **16**, collection membranes **14**, collection membrane supports **20**, and/or base **12** together. Covers **18** are reversibly adhered or attached to separation membranes **16**, collection membranes **14**, collection membrane supports **20**, and/or base **12** by any suitable means. For example, covers **18** can be stickers having an adhesive side that is used to attach covers **18** to separation membranes **16**, collection membranes **14**, collection membrane supports **20**, and/or base **12**. Covers **18** are reversibly adhered so that covers **18** are easily removed in order to access and remove collection membranes **14** and/or separation membranes **16** from separation device **10** for further processing and analysis.

[0055] Covers **18** can include one or more projections **30** that make removal of covers **18** easier. For example, covers **18** can have a graspable tab to facilitate removal of covers **18** from separation device **10**.

[0056] Separation device **10** can also include a desiccant for drying, or keeping dry, a biological specimen. The desiccant can be disposed anywhere on separation device **10** (e.g. on or in base **12**).

-15-

[0057] Separation device **10** can include one or more caps **32**. Caps **32** generally have one or more sample deposition apertures **28** therethrough that define an area for depositing a liquid biological specimen. Caps **32** can operate to removably secure the separation membranes **16**, collection membranes **14**, and/or base **12** together. Caps **32** can be reversibly attached to base **12** by any suitable means. For example, separation device **10** can include elastomeric band fasteners **38** that urge and hold base **12** and caps **32** together. As another example, base **12** can be secured to cap **32** by a twist-lock mechanism **54** that urges and holds base **12** and caps **32** together. Caps **32** can be made of, for example, suitable plastics materials (e.g. polyethylene, acrylic, polypropylene).

10 [0058] FIGS **1A-2** show a liquid biological specimen separation device **10** in accordance with one embodiment of the invention. The liquid biological specimen separation device **10** has a base **12**. Base **12** is a flat bifacial sheet having a uniform thickness and a modified cinquefoil shape defined by a series of radial projections extending therefrom. The largest radial projection is a handle that facilitates
15 handling/holding separation device **10**. Each of the four remaining radial projections provide a supporting surface or region for a corresponding one of the biological specimen receiving/separation/collection regions.

[0059] Disposed on liquid biological specimen separation device **10** are four circular collection membrane supports **20** and corresponding circular collection
20 membranes **14** disposed therein. Each of the four collection membrane supports **20** is separated from a corresponding collection membrane **14** by perforations, the perforations enabling easy removal of the collection membranes **14** from their respective collection membrane support **20**. Each of the collection membrane supports **20** also includes two removal apertures **26** that enable the use of a device (e.g. tweezers or tongs) to selectively
25 grasp and remove collection membranes **14** from their respective collection membrane support **20**.

[0060] The liquid biological specimen separation device **10** has four circular separation membranes **16**, each of which is disposed on a respective one of the collection membranes **14** and collection membrane supports **20**. The circular separation membranes
30 **16**, when disposed on collection membranes **14**, completely overlap the collection membranes **14** because the diameter of separation membranes **16** is larger than the

-16-

diameter of collection membranes **14**. The diameter of separation membranes **16**, however, is smaller than the diameter of collection membrane supports **20**.

[0061] The liquid biological specimen separation device **10** has four generally circular covers **18**, each having a tab extending therefrom. The covers **18** are stickers, and
5 each sticker is adhesively disposed on a respective one of the separation membranes **16** and collection membrane supports **20**. Each sticker has a biological sample deposition aperture extending therethrough that permits depositing a liquid biological specimen onto the separation membrane **16** secured underneath the sticker.

[0062] FIGS 3A-3B show a liquid biological specimen separation device **10** in
10 accordance with another embodiment of the invention. The liquid biological specimen separation device **10** of FIGS 3A-3B is substantially the same as the liquid biological specimen separation device **10** of FIGS 1A-2 except that base **12** is a semi-circular shape having one radial projection extending therefrom, the radial projection serving as a handle for handling separation device **10**.

15 [0063] FIGS 4A-5 show a liquid biological specimen separation device **10** in accordance with another embodiment of the invention. The liquid biological specimen separation device **10** of FIGS 4A-5 is similar to the liquid biological specimen separation device **10** of FIGS 1A-2 with a few differences. As shown in FIGS 4A-5, separation device **10** has one collection membrane support **20** and one cover **18**. The collection
20 membrane support **20** and cover **18** are generally shaped to match the portion of base **10** having the four radial projections. Cover **18** also has two tabs extending therefrom and projecting outwards from separation device **10**.

[0064] FIGS 6-8 show a liquid biological specimen separation device **10** in accordance with another embodiment of the invention. In this embodiment, base **12** is a
25 generally square shape having four radial lobes projecting outwards from separation device **10**, one projection at each corner. The liquid biological specimen separation device **10** includes one collection membrane **14**, one collection membrane support **20**, one separation membrane **16**, and one cover **18**.

[0065] FIGS 9A-9D show a liquid biological specimen separation device **10** in
30 accordance with another embodiment of the invention. The liquid biological specimen separation device **10** has a base **12**. Base **12** is made of a clear plastic material and has a

-17-

flip top design. Base **12** has four wells **30** and four corresponding caps **32** that are designed to complementary mate with one another. Caps **32** are connected to the rest of base **12** by arms **34**. Arms **34** have a hinge **36** that allows movement of caps **32** relative to wells **30**. Base **12** can assume a first position wherein caps **32** extend away from wells **30** (e.g. an open configuration, as shown). Base **12** can assume a second position wherein caps **32** and wells **30** complementary mate with one another to form a closed configuration. Each cap **32** includes a sample deposition aperture **28** therethrough that defines an area for depositing a liquid biological specimen. Each cap **32** also includes a tab feature that facilitates opening separation device **10** when in a closed configuration.

10 [0066] Disposed in wells **30** of liquid biological specimen separation device **10** are four circular collection membrane supports **20**. Circular collection membranes **14** (not shown) are disposable in a respective one of the collection membrane supports **20**. Each of the collection membrane supports **20** includes two removal apertures **26** that enable the use of a device (e.g. tweezers or tongs) to selectively grasp and remove collection
15 membranes **14** from their respective collection membrane support **20**.

[0067] Four circular separation membranes **16** (not shown) are disposable in a respective one of the recesses in caps **32**. Four generally circular covers **18** (not shown) are disposable on a respective one of the separation membranes **16**. The covers **18** are stickers, and each sticker is adhesively disposed on a respective one of the separation
20 membranes **16** and an internal surface of caps **32**. Each sticker adheres a separation membrane **16** to an internal surface of a respective cap **32**. Each sticker has a biological sample deposition aperture extending therethrough that permits flow from separation membrane **16** to collection membrane **14**. In a closed configuration of separation device **10**, at least a portion of separation membrane **16** comes into contact with collection
25 membrane **14** (some contact between the two membranes can be intermediated by a cover **18**).

[0068] FIGS 10A-10B show a liquid biological specimen separation device **10** in accordance with another embodiment of the invention. The liquid biological specimen separation device **10** has a base **12**. Base **12** is made of a plastic material. Base **12** has a
30 three dimensional shape comprising a relatively low profile outer periphery **40** and a raised inner projection **42**. The raised inner projection **42** has a first recess **44** therein dimensioned to receive a separation membrane **16**. The first recess **44** has a second recess

-18-

46 therein dimensioned to receive a collection membrane **14**. Base **12** also has two fastening tabs **50** extending therefrom.

[0069] The liquid biological specimen separation device **10** has a cap **32**. The cap **32** is dimensioned to complementary mate with raised inner projection **42**. Cap **32** includes a funnel portion arranged to direct fluid flow towards a sample deposition aperture **28** extending through cap **32**, the sample deposition aperture **28** defining an area for depositing a liquid biological specimen. Cap **32** has two diametrically opposing pegs **48** extending therefrom. Pegs **48** are positioned on cap **32** such that they are alignable with fastening tabs **50**.

10 [0070] Disposed in the second recess **46** is a collection membrane **14**. The second recess **46** and collection membrane **14** can be dimensioned such that collection membrane **14** sits flush with a top surface of the first recess **44**. The second recess **46** and collection membrane **14** can be dimensioned such that that collection membrane **14** extends a short distance (e.g. 0.5 mm) above a top surface of the first recess **44**. In either case, at least a
15 portion of separation membrane **16** comes into contact with collection membrane **14**.

[0071] Disposed in the first recess **44** is a separation membrane **16**. Separation membrane **16**, collection membrane **14**, and cap **32** are aligned about an axis extending through a center point of each of the separation membrane **16**, collection membrane **14**, and cap **32**.

20 [0072] The liquid biological specimen separation device **10** has two fasteners **38**. Fasteners **38** are elastomeric bands wrappable around pegs **48** and fastening tabs **50** such that base **12** is secured to cap **32**.

[0073] The liquid biological specimen separation device **10** has at least a closed configuration and an open configuration.

25 [0074] In a closed configuration, fasteners **38** are wrapped around pegs **48** and fastening tabs **50**, thereby securing base **12** to cap **32**. Contained in between base **12** and cap **32** is collection membrane **14**, which is disposed in the second recess **46**, and separation membrane **16**, which is disposed in the first recess **44**. Fasteners **38** urge cap **32** towards base **12** with sufficient force such that cap **32** collapses pores in separation
30 membrane **16**, thereby forming a liquid impermeable barrier **52** in between two regions of separation membrane **16**. The liquid impermeable barrier **52** is dimensioned to be slightly

-19-

larger in width (e.g. radius) than collection membrane **14**, thereby minimizing the volume of plasma lost to lateral leakage and/or absorption by separation membrane **16**.

[0075] In an open configuration, fasteners **38** are not wrapped around pegs **48** and fastening tabs **50**, thereby allowing disassembly of the liquid biological specimen separation device **10**. For example, collection membrane **14** and separation membrane **16** are removable from liquid biological specimen separation device **10** for use in various analytic techniques.

[0076] Sample deposition aperture **28** can have a diameter/width of 8.3 to 9.2 mm. That is, sample deposition aperture **28** can have a diameter/width of 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, or 9.2 mm.

[0077] Collection membrane **14** can have a diameter/width of 3 to 10.5 mm and a thickness of 0.19 mm to 0.270 mm.

[0078] First recess **44** can have a diameter/width of 10 to 25 mm.

[0079] Separation membrane **16** can have a diameter/width of 9 to 11 mm and a thickness of 0.19 mm to 0.380 mm.

[0080] Second recess **46** can have a diameter/width that is 0.1 to 0.5 mm larger than a diameter/width of collection membrane **14**, and preferably has a diameter/width that is smaller than the sample deposition aperture **28**.

[0081] Liquid impermeable barrier **52** can have a diameter/width that is 0.6 to 1.0 mm larger than a diameter of sample deposition aperture **28**.

[0082] FIGS 11A-11F show a liquid biological specimen separation device **10** in accordance with another embodiment of the invention. The liquid biological specimen separation device **10** of FIGS 11A-11F is substantially the same as the liquid biological specimen separation device **10** of FIGS 10A-10B except that base **12** is secured to cap **32** by a twist-lock mechanism **54** rather than fasteners **38**, pegs **48**, and fastening tabs **50**. Base **12** and cap **32** each have complementary fastening protrusions **56** with ridge features **58**. The complementary fastening protrusions **56** of base **12** and cap **32** can be engaged to secure base **12** to cap **32** and disengaged to unsecure base **12** from cap **32**, respectively, by rotating base **12** relative to cap **32**. When the complementary fastening protrusions **56** of base **12** and cap **32** are engaged with one another, cap **32** is urged towards base **12** with

-20-

sufficient force such that cap **32** collapses pores in separation membrane **16**, thereby forming a liquid impermeable barrier **52** in between two regions of separation membrane **16**.

[0083] In operation, to secure cap **32** to base **12**, complementary fastening protrusions **56** on cap **32** can be inserted into apertures **60** in base **12** that are dimensioned to receive complementary fastening protrusions **56**. Cap **32** is then rotated relative to base **12** such that complementary fastening protrusions **56** and ridge features **58** on both base **12** and cap **32** are aligned and engaged. The reverse operations can be performed to unsecure cap **32** from base **12**.

10 I. Applications and Use

[0084] Methods of using a liquid biological specimen separation device are provided. Generally, a liquid biological specimen separation device is used to receive a liquid biological specimen containing an analyte of interest, separate the liquid biological specimen into two components, and store an analyte of interest. The liquid biological specimen separation device is suitable for use as a point-of-care device.

[0085] In certain embodiments, the methods include providing a liquid biological specimen separation device and dispensing a liquid biological specimen onto a separation membrane of the device via an aperture in a cover and/or a cap. The liquid biological specimen flows through the separation membrane (e.g. via capillary action, gravity, etc).
20 A first component of the liquid biological specimen is trapped/retained by the separation membrane while a second component of the liquid biological specimen flows through the separation membrane and into the collection membrane, which absorbs the second component. The second component can be dried in the collection membrane, either actively (e.g. via a desiccant or heating) or passively (e.g. air dry), before further
25 processing. Alternatively, the second component can be used for further processing prior to being dried out (e.g. while still wet). The separation membrane and/or the collection membrane having the first and second components respectively can be removed from the device and be exposed to or placed in a reconstitution media to remove/recover analytes of interest therefrom, which can then be analyzed using a suitable technique for the analyte to
30 be studied. In certain embodiments, the methods can include compressing the collection and/or separation membranes to aid in recovering analytes of interest therefrom. In certain

-21-

embodiments, the methods can include applying reconstitution media to the separation and/or collection membranes to rehydrate analytes of interest contained therein, and compressing the membranes to release the analytes of interest. In certain embodiments, the separation devices and methods separate a liquid biological specimen into at least two constituent components that are subsequently air-dried and stored at ambient temperatures (e.g. without the need for refrigeration or freezing) prior to subsequent quantitative and qualitative analysis on at least one analyte of interest in at least one of the components.

[0086] In certain embodiments, the methods include providing a liquid biological specimen separation device. The methods can include adding approximately 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 uL of whole blood from either a pipette or directly from a patient's finger-stick or heel-stick onto a top surface of a separation membrane of the device via an aperture in a cover and/or a cap. In embodiments, disposing 20-200 uL of whole blood onto a top surface of a separation membrane of the device results in 5-50 uL of plasma saturating a collection membrane. In embodiments, the volume of plasma is equal to about one-quarter the volume of whole blood. The methods can include adding approximately 25-40 uL of whole blood from either a pipette or directly from a patient's finger-stick or heel-stick onto a top surface of a separation membrane of the device via an aperture in a cover and/or a cap. In embodiments, disposing 25-40 uL of whole blood onto a top surface of a separation membrane of the device results in 6-10 uL of plasma saturating a collection membrane.

[0087] The methods can include having a user wait approximately 1, 2, 3, 4, 5, or 10 or more minutes for transfer of plasma through the separation membrane to the collection membrane (e.g. via capillary action, gravity, etc). The methods can include having a user allow the collection membrane to dry (e.g. placing the collection membrane in a designated air drying location) for 1, 2, 3, 4, 5, 6, 12, 24, or more hours. The drying can be accomplished at room temperature by air drying or at controlled temperature. The methods can include placing the whole device or the dried collection membrane in a sealable packaging, which can include a desiccant, for shipment to a laboratory for further analysis. The methods can include the laboratory separating the collection membrane from the separation device. The methods can include the laboratory separating white blood solids from the collection membrane. The methods can include the laboratory suspending the collection membrane in a reconstitution medium.

-22-

[0088] In certain embodiments, the reconstitution medium is molecular-grade water. In other embodiments, the reconstitution medium includes nuclease-free water or the components of phosphate buffered saline (PBS) or other suitable buffered saline solutions. Optionally, the reconstitution medium includes sodium azide or other antimicrobial agents. The reconstitution medium can also include any number or combinations of available biological preservatives or blood anticoagulants including but not limited to ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin. Saline solutions or nuclease-free water can serve as a sterile and neutral medium for the rehydration, re-suspension, and recovery of analyte(s) of interest from the collection and/or separation membranes. When included, antimicrobial agents such as sodium azide prevent microbial growth and subsequent contamination with RNases. When included, biological preservatives such as EDTA, sodium citrate, and heparin serve as anticoagulants and or chelating agents.

[0089] The volume of a membrane may or may not expand upon absorption of a liquid biological specimen, and may or may not contract upon drying. However, a liquid saturated membrane can be compressed to release entrained fluid containing an analyte of interest, due to its porosity, by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or more of its saturated volume. Volumetric compression is one convenient technique for releasing analytes of interest, however, other means such as centrifugation or vacuum pressure can alternatively be employed to release analytes of interest from a membrane.

[0090] In certain embodiments, the methods can also include an intermediate step of applying a stabilizing composition to the collection membrane and/or the separation membrane to protect analytes of interest against degradation. Depending upon the analytes of interest, the stabilizing composition may include one or more of a weak base, a chelating agent, a protein denaturing agent such as a detergent or surfactant, a nuclease inhibitor, and a free radical trap. Particularly for protection of unstable RNA, the stabilizing composition may include RNase inhibitors and inactivators, genetic probes, complementary DNA or RNA (or functionally equivalent compounds), proteins and organic moieties that stabilize RNA or prevent its degradation.

-23-

[0091] In embodiments, the time periods for which analytes of interest may be preserved or stored on a collection and/or separation membrane can be for a period of several minutes, hours, days, months, or even greater.

[0092] Temperature conditions under which analytes of interest may be preserved
5 or stored on a collection and/or separation membrane are not limited. Typically, analytes of interest are kept at ambient or room temperature, for example, from about 15°C to about 40°C, preferably from about 15°C to about 25°C. In some embodiments, the analytes of interest may be kept in a cool environment. For example, in short-term storage, the analytes can be refrigerated at about 2°C to about 10°C. In yet another example, the
10 analytes may be refrigerated at about 4°C to about 8°C. In another example, in long-term storage, the analytes can be frozen at about -20°C to about -80°C. In addition, the membranes may preferably, but not necessarily, be stored in dry or desiccated conditions or under an inert atmosphere.

[0093] In certain embodiments, whole blood is dispensed onto a liquid biological
15 specimen separation device. In such embodiments, whole blood or a liquid suspension thereof is deposited onto a separation membrane. The separation membrane absorbs the whole blood. The separation membrane captures some solid components of whole blood (e.g., WBCs, RBCs, platelets, and/or other cellular components) while allowing fluidic and/or other solid whole blood components (e.g. cell-free plasma) to pass through the
20 separation membrane via gravity and/or capillary action. The components of whole blood passing through the separation membrane are absorbed by the collection membrane.

[0094] In certain embodiments, cell-free plasma captured on the collection
membrane can be removed/recovered from the collection membrane by exposing the collection membrane to a reconstitution media. The recovered cell-free plasma can
25 contain an analyte of interest, for instance, nucleic acids such as DNA and RNA, which can be used for viral load quantitation, genotyping, drug resistance testing, or other suitable analyses. The analytes of interest can be detected or analyzed using analytical and diagnostic methods known in the art.

[0095] The detailed description set forth above is provided to aid those skilled in
30 the art in practicing the invention. However, the invention described and claimed herein is not to be limited in scope by the specific embodiments described above, as these

-24-

embodiments are presented as mere illustrations of several aspects of the invention. Any combinations and modifications of the described methods and components, and compositions used in the practice of the methods, in addition to those not specifically described, will become apparent to those skilled in the art based on the present disclosure
5 and do not depart from the spirit or scope of the present invention. Such variations, modifications, and combinations are also encompassed by the present disclosure and fall within the scope of the appended claims.

-25-

WHAT IS CLAIMED IS:

1. A liquid biological specimen separation device comprising:
 - a base;
 - a collection membrane disposed on the base,
 - a separation membrane disposed on the collection membrane, wherein the separation membrane comprises a polysulfone polymer material selected from the group consisting of asymmetric sub-micron polysulfone and asymmetric super micron polysulfone; and
 - a cap disposed on the separation membrane and the base, wherein the cap comprises an aperture therein configured to allow deposition of a liquid biological specimen therethrough.
2. The device of claim 1, wherein the separation membrane has a porosity that gradually decreases from a first side to a second side so as to filter and trap solid components of a liquid biological specimen deposited on the separation membrane.
3. The device of claim 1, wherein the separation membrane is configured to filter and trap solid components of a biological specimen, the biological specimen being selected from the group consisting of whole blood, plasma, urine, saliva, sputum, semen, vaginal lavages, bone marrow and cerebrospinal fluid.
4. The device of claim 1, wherein the separation membrane is configured to filter and trap solid components of a whole blood specimen, and wherein the collection membrane is configured to separately filter and trap a plasma fraction or filtrate of the whole blood specimen.
5. The device of claim 1, wherein the separation membrane has a pore size ranging from 0.1-20 μm .
6. The device of claim 1, wherein the collection membrane comprises a substantially hydrophobic polyolefin material comprising a plurality of polypropylene fibers coated with hydrophobic polyethylene.

-26-

7. The device of claim 1, wherein the collection membrane, the separation membrane, or both, comprise microglass fibers.
8. The device of claim 1, wherein the cap removably adheres to the base.
9. The device of claim 8, wherein the cap removably adheres to the base by a twist-lock mechanism.
10. The device of claim 8, further comprising at least two elastomeric band fasteners, and wherein the cap removably adheres to the base by the at least two elastomeric band fasteners.
11. The device of claim 1, wherein the cap is urged towards the base.
12. The device of claim 11, wherein the separation membrane comprises a liquid impermeable barrier between two regions of the separation membrane formed from collapsed pores in the separation membrane as a result of the urging.
13. The device of claim 1, further comprising an identifier disposed on the base.
14. The device of claim 1, wherein the base comprises a first recess and a second recess.
15. The device of claim 14, wherein the first recess and second recess are dimensioned to receive the separation membrane and the collection membrane, respectively.
16. The device of claim 14, wherein the second recess is disposed in the first recess.
17. The device of claim 1, wherein the aperture, the separation membrane, and the collection membrane are aligned about an axis extending through a center point of each of the aperture, the separation membrane, and the collection membrane.
18. A method for separating plasma from whole blood comprising:
providing a device according to claim 1; and
depositing whole blood through the aperture and onto the separation membrane,
19. The method of claim 18, wherein 25-40 uL of whole blood is deposited onto the separation membrane.

-27-

20. The method of claim 18, wherein 6-10 uL of plasma are recovered in the collection membrane.

1/16

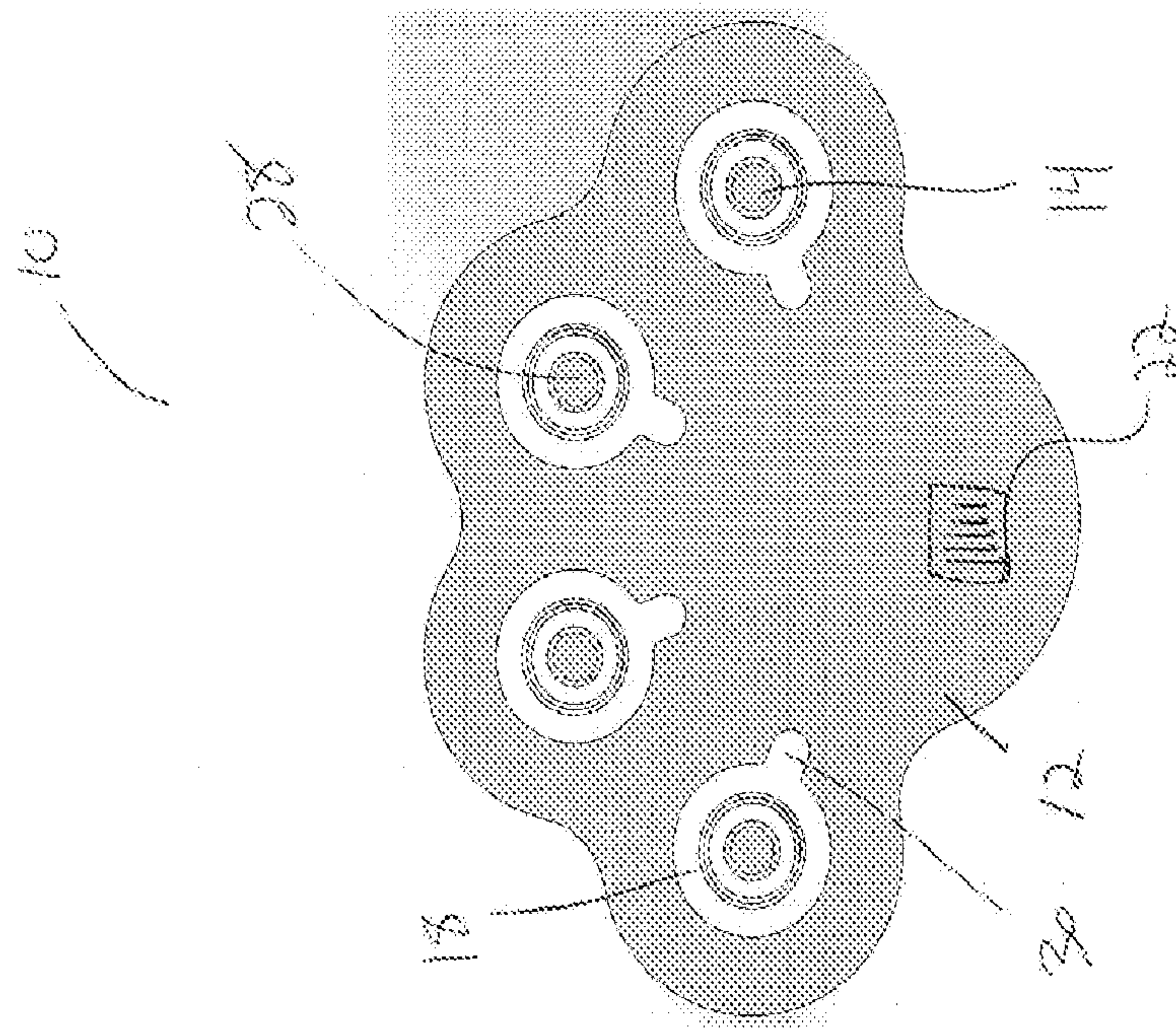


Fig. 1B

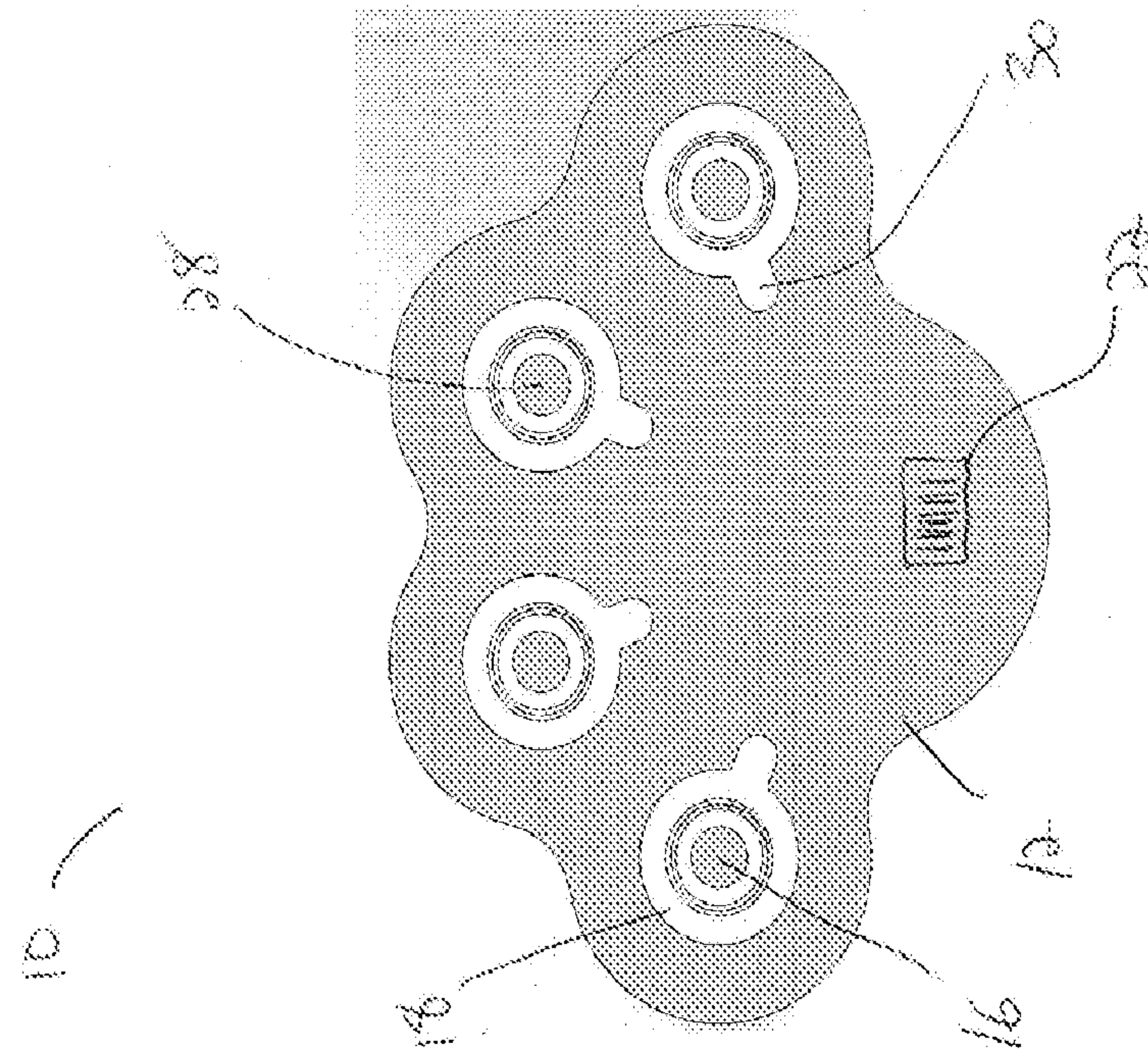
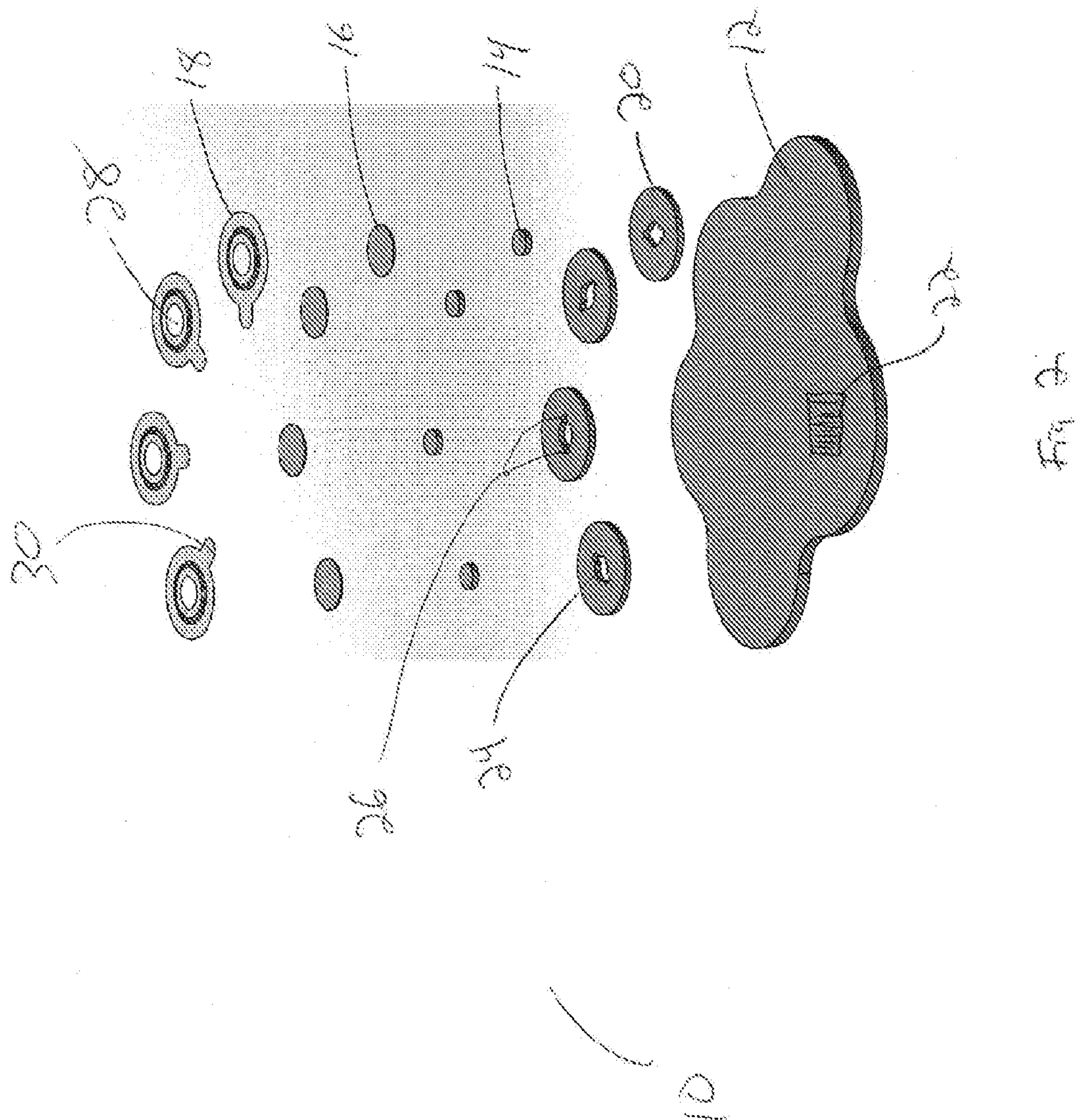


Fig. 1A

2/16



3/16

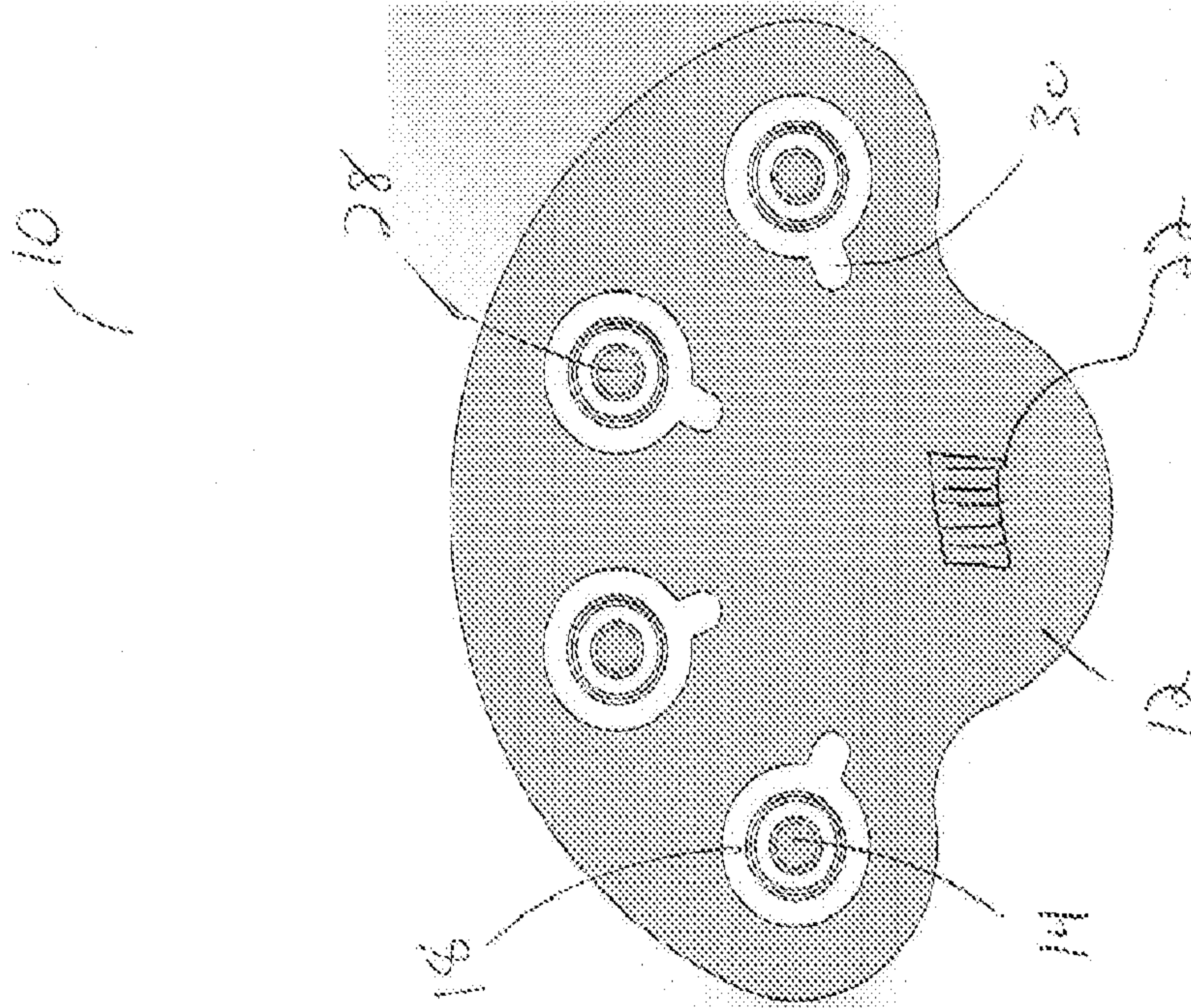


Fig. 3B

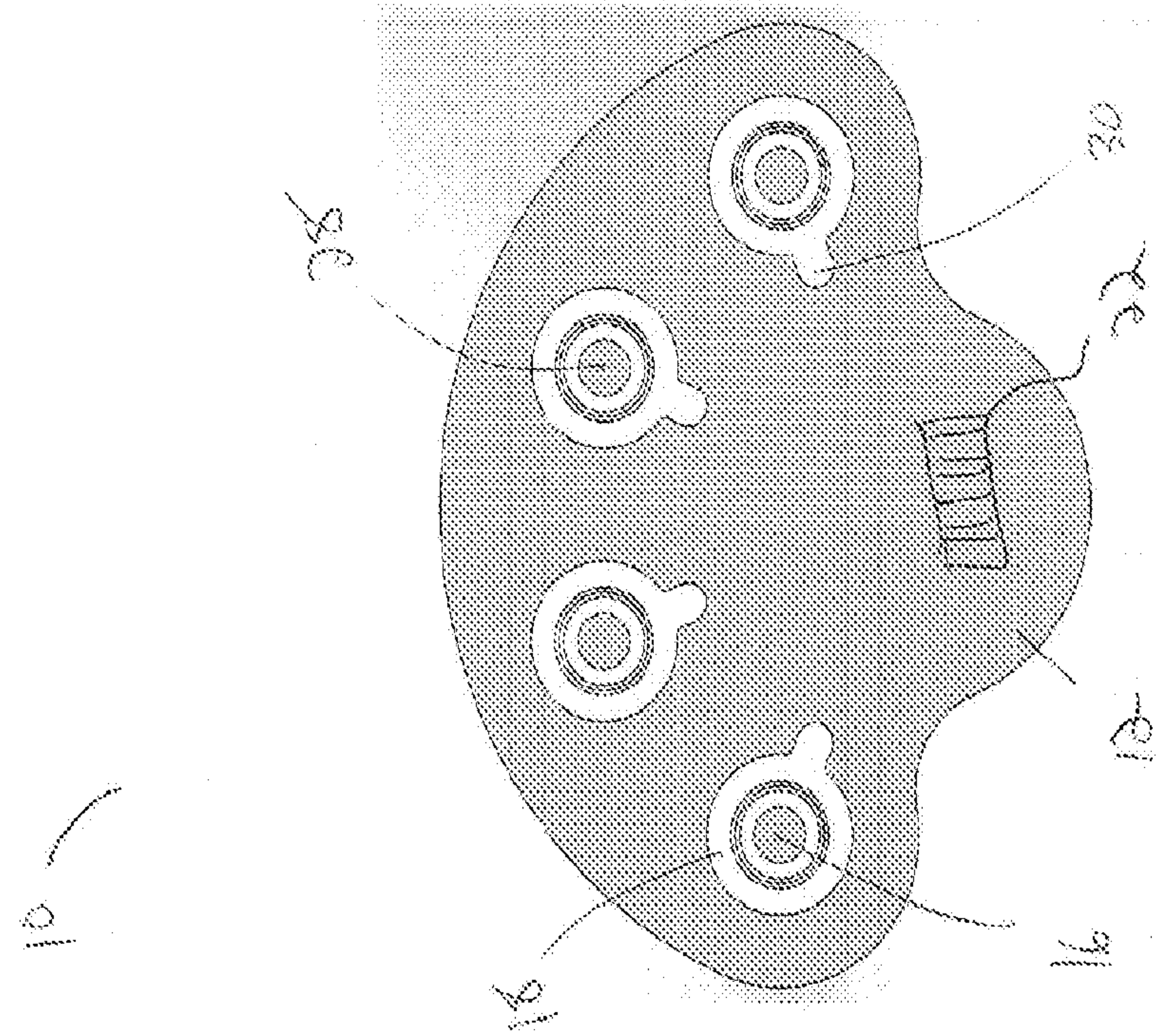


Fig. 3A

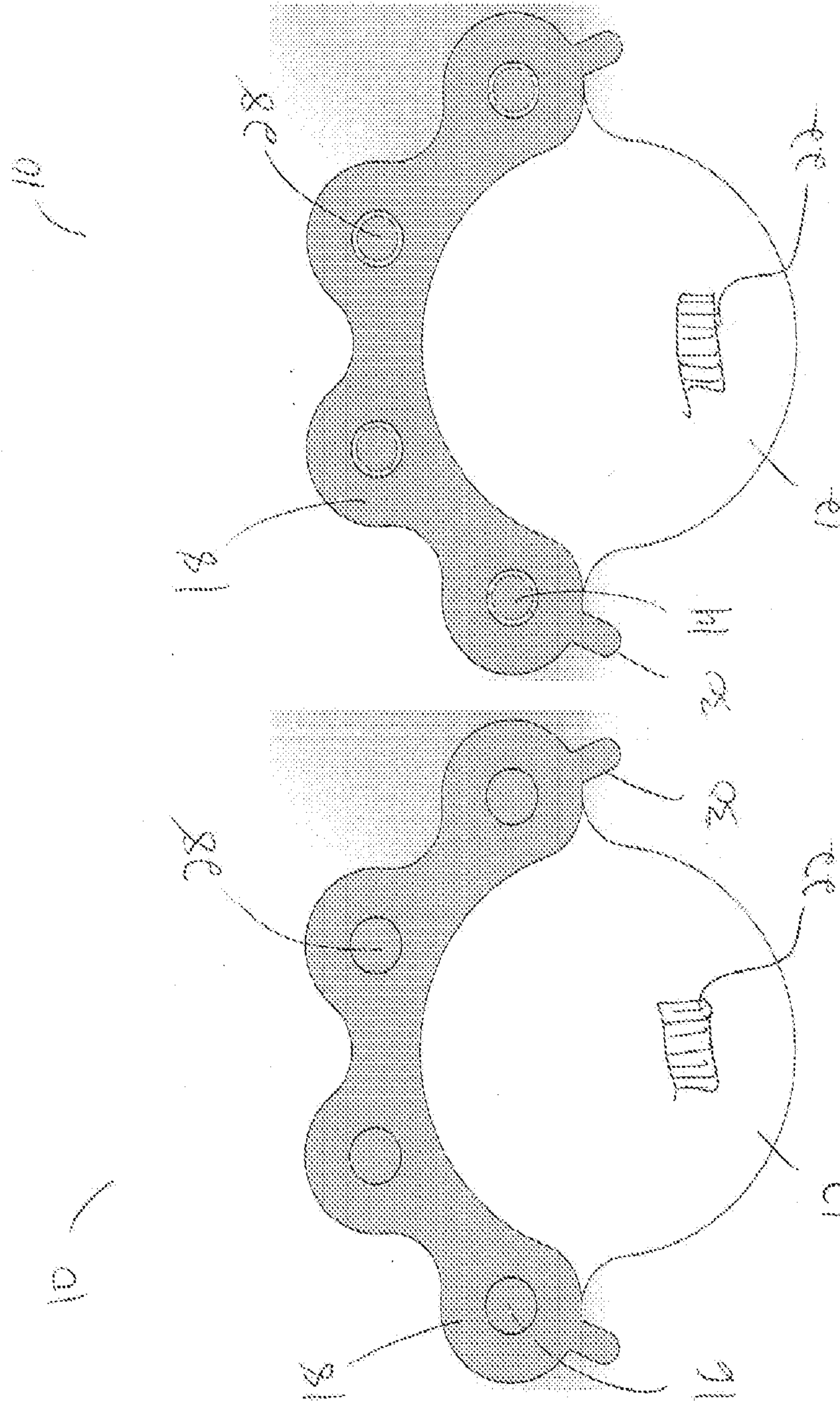


Fig. 48

Fig. 44

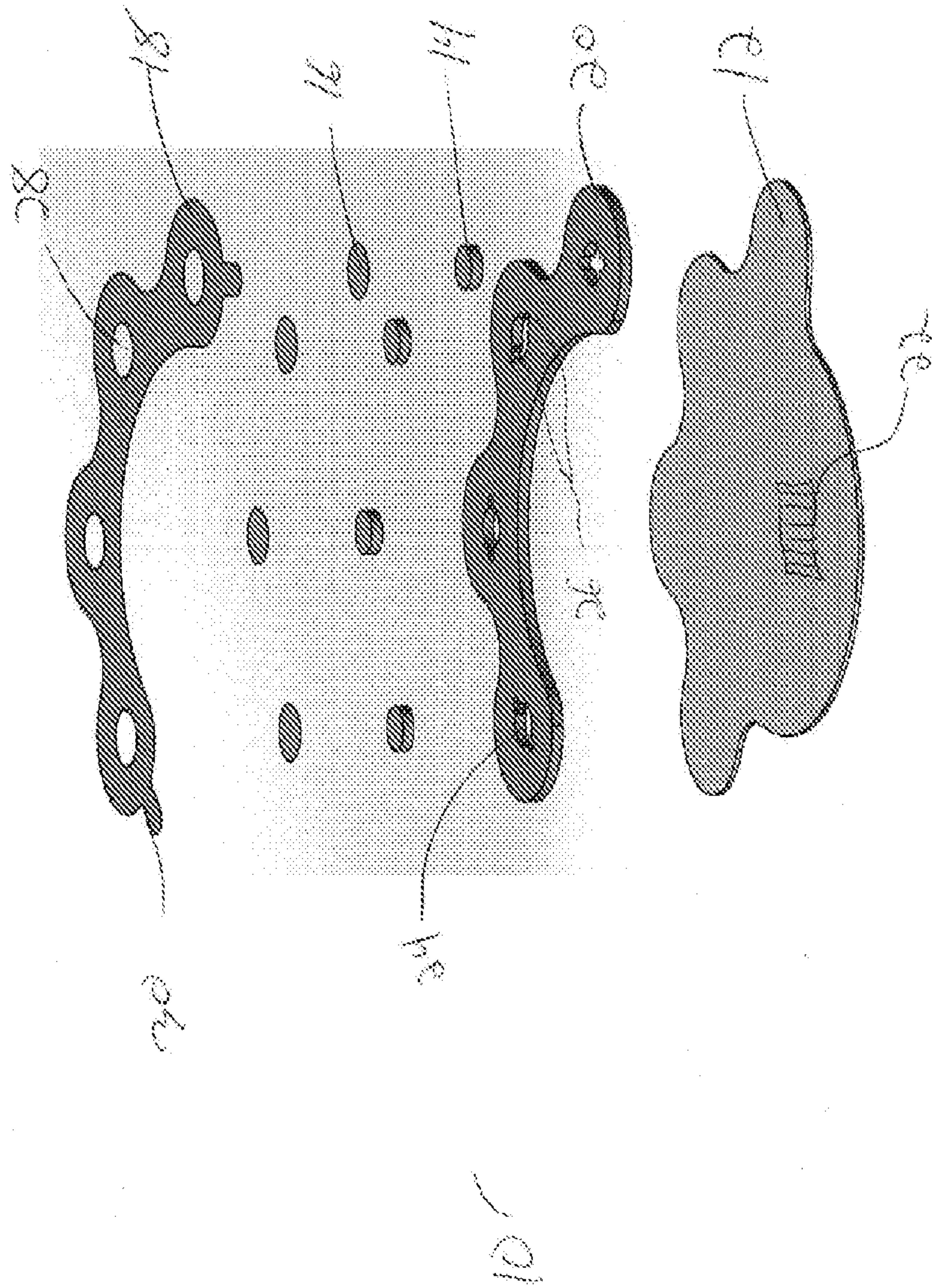
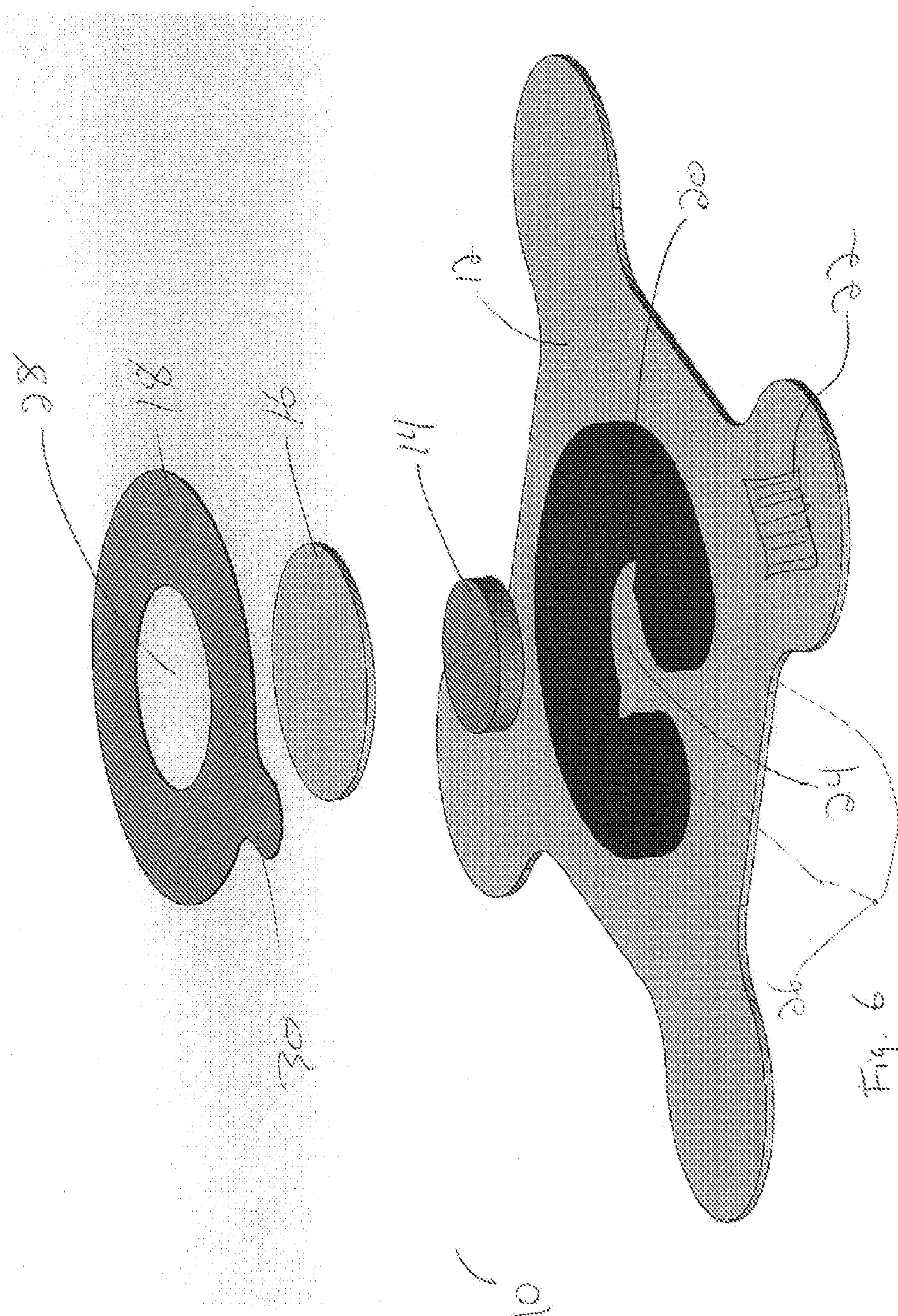


Fig. 5



7/16

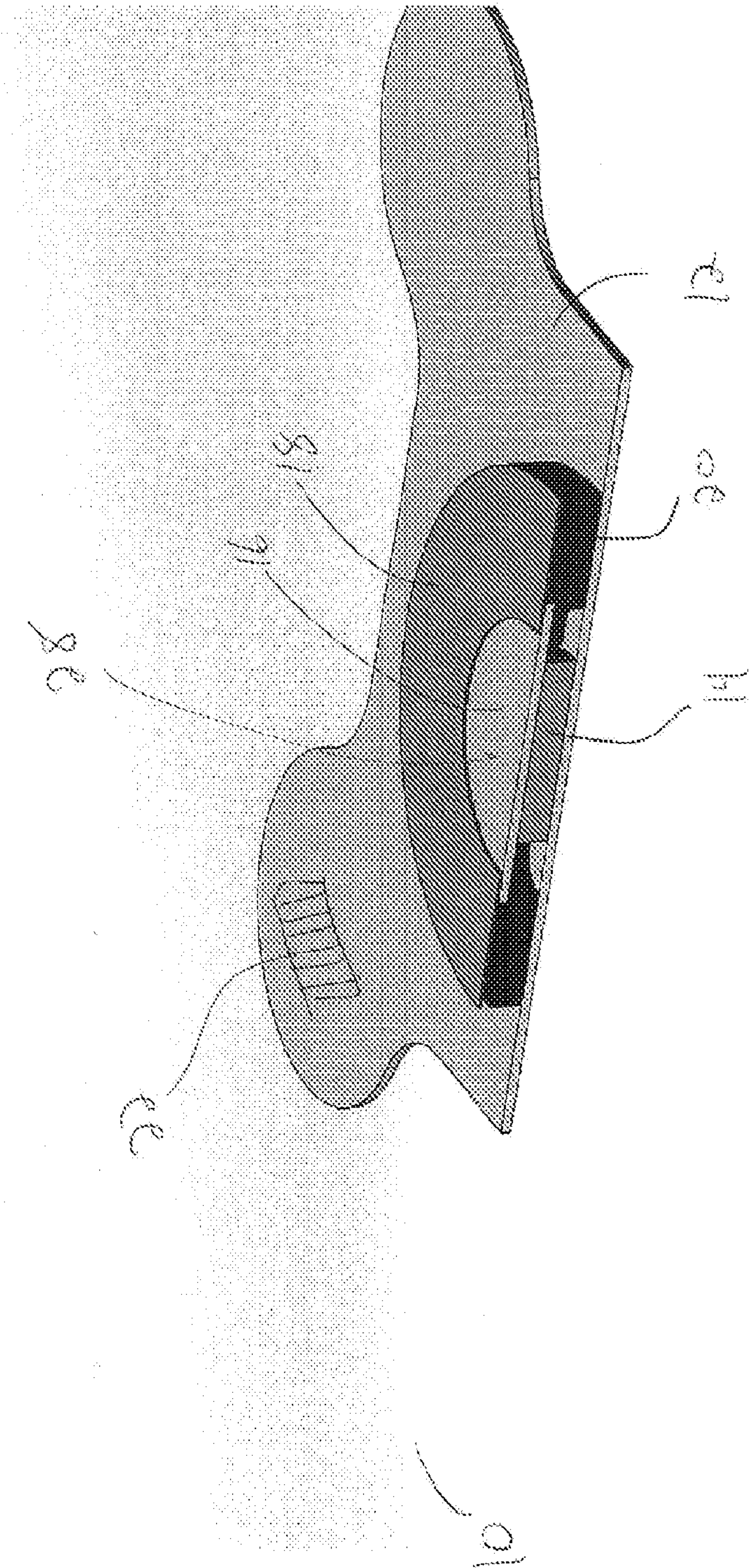


Fig. 7

8/16

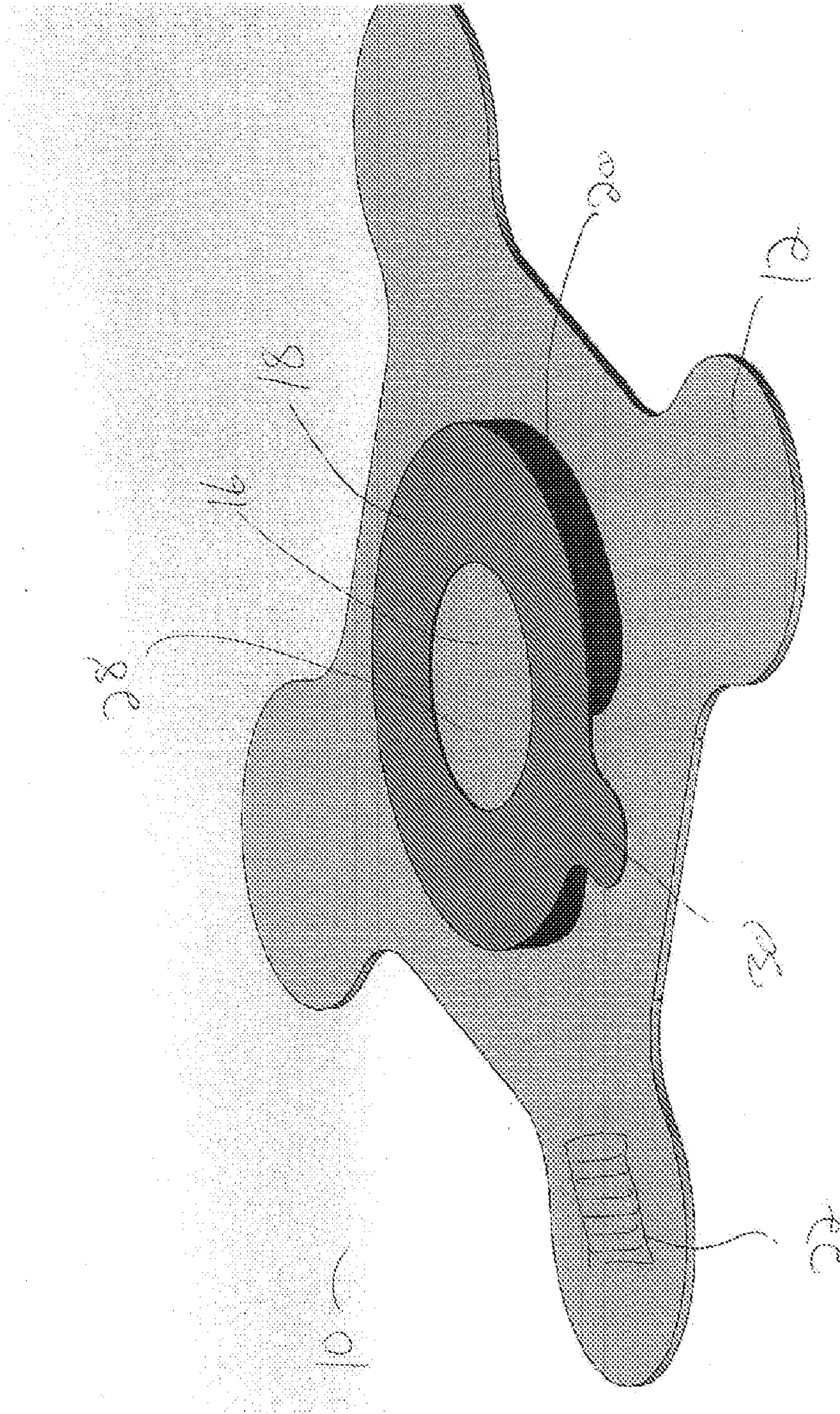
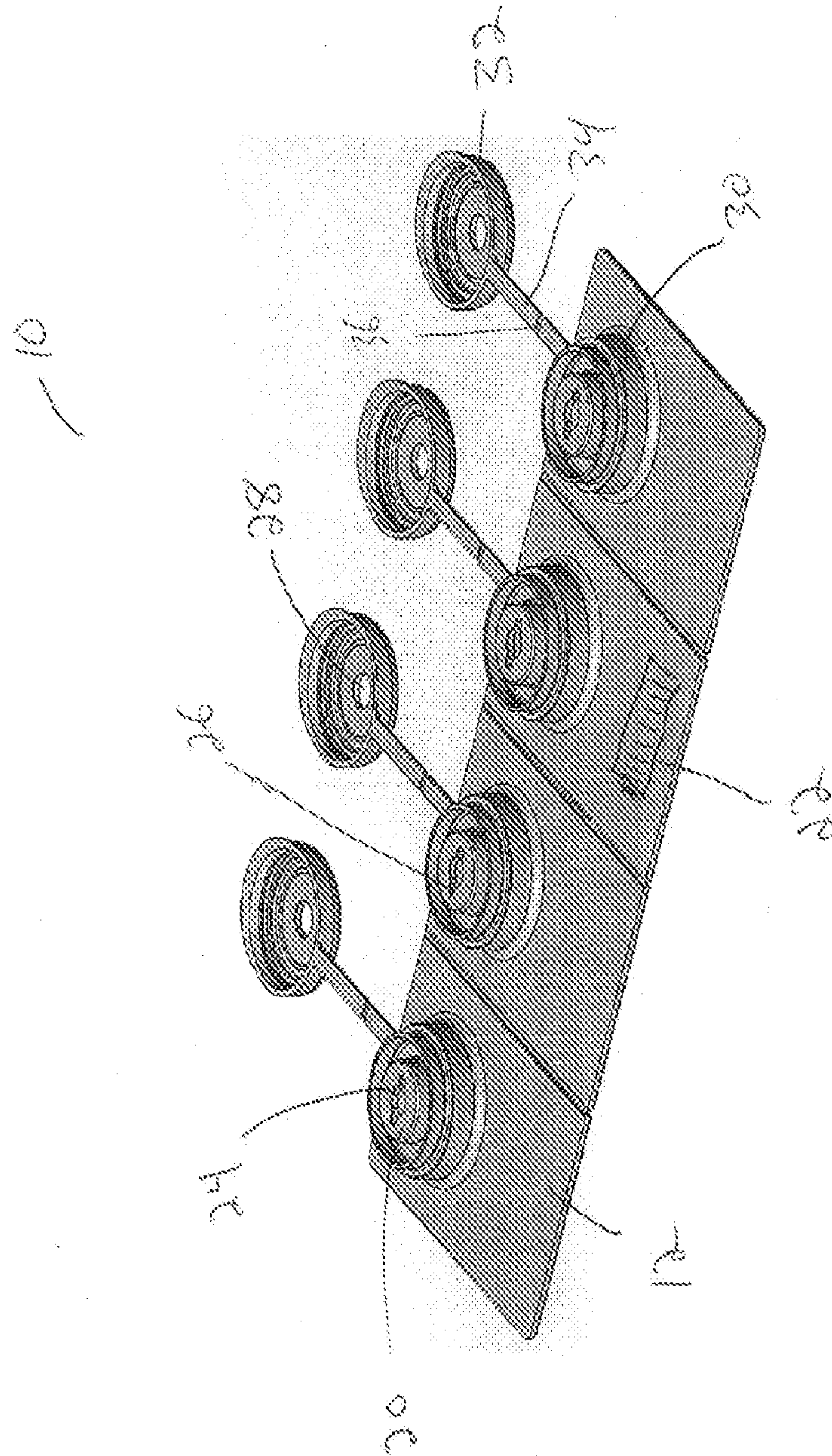
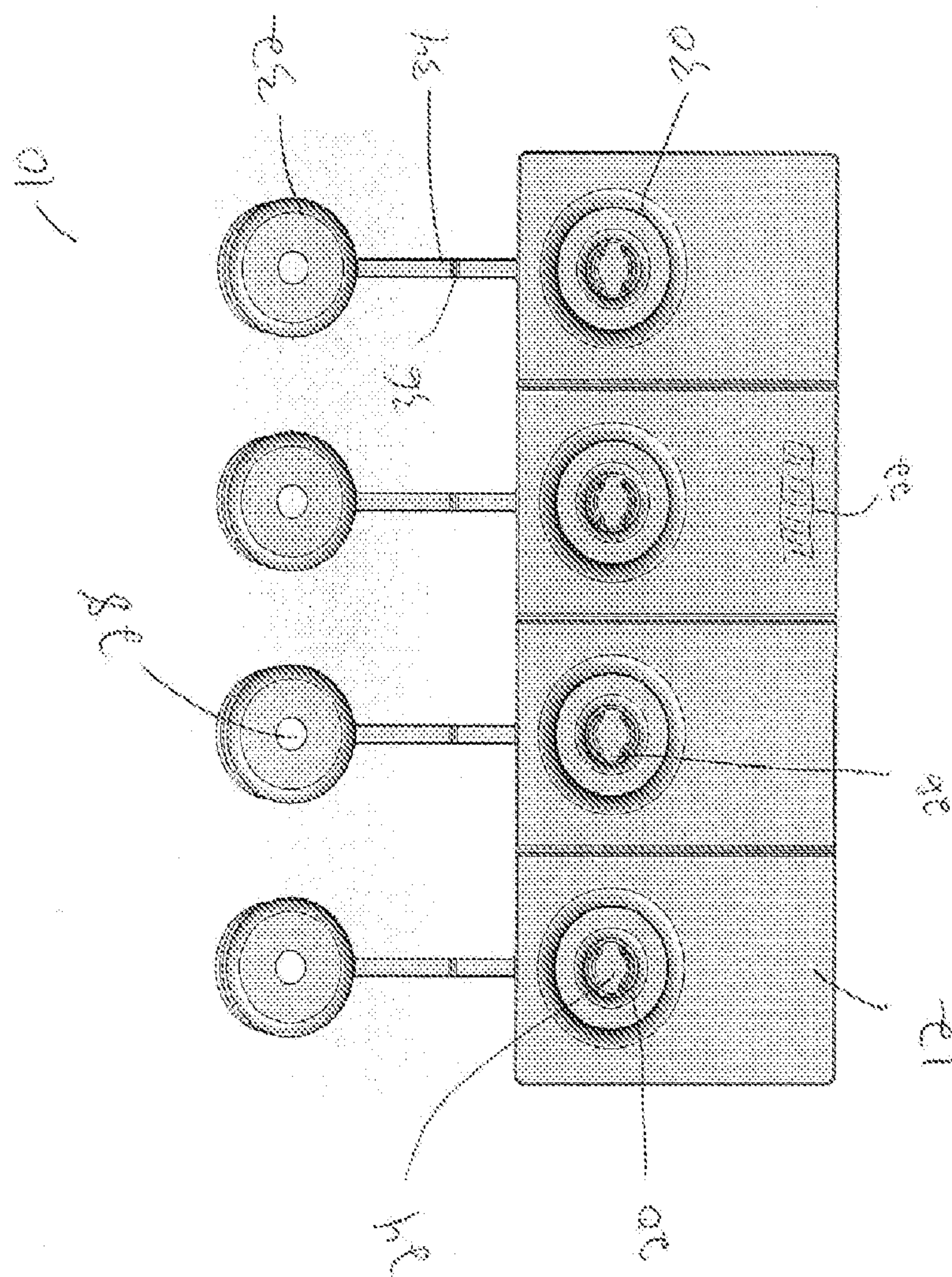


Fig. 8

9/16





11/16

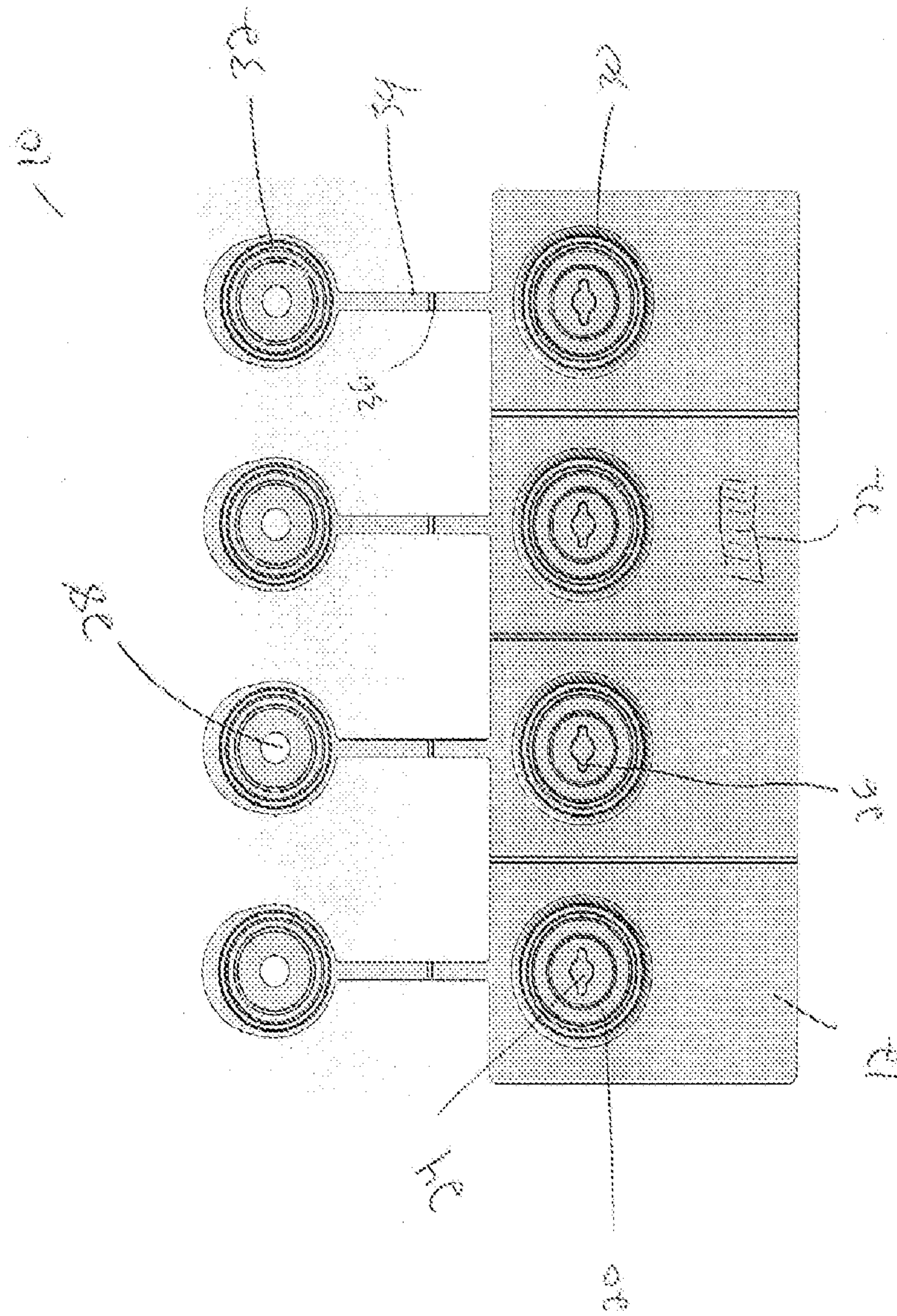
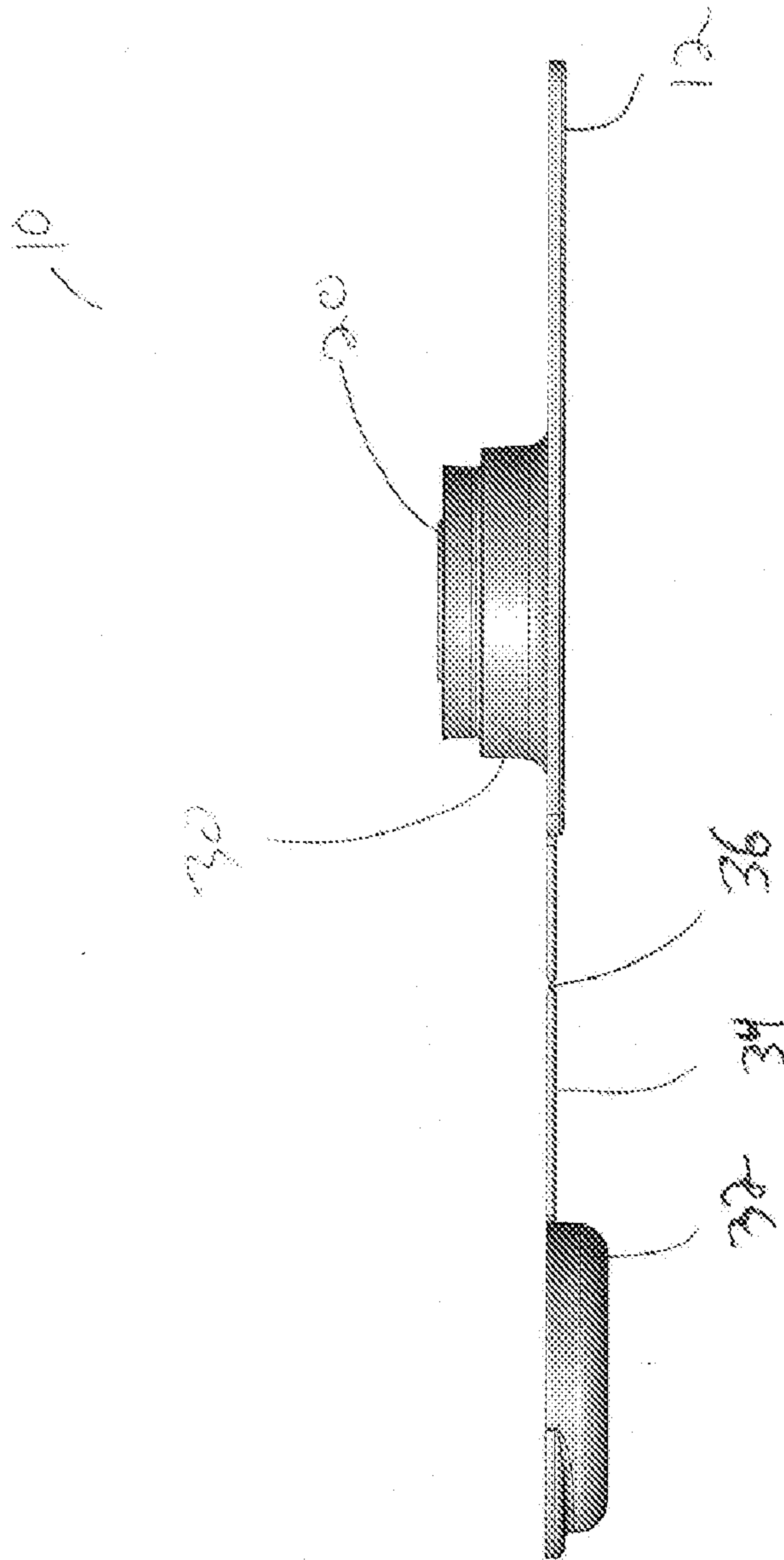


FIG. 9c

12/16



13/16

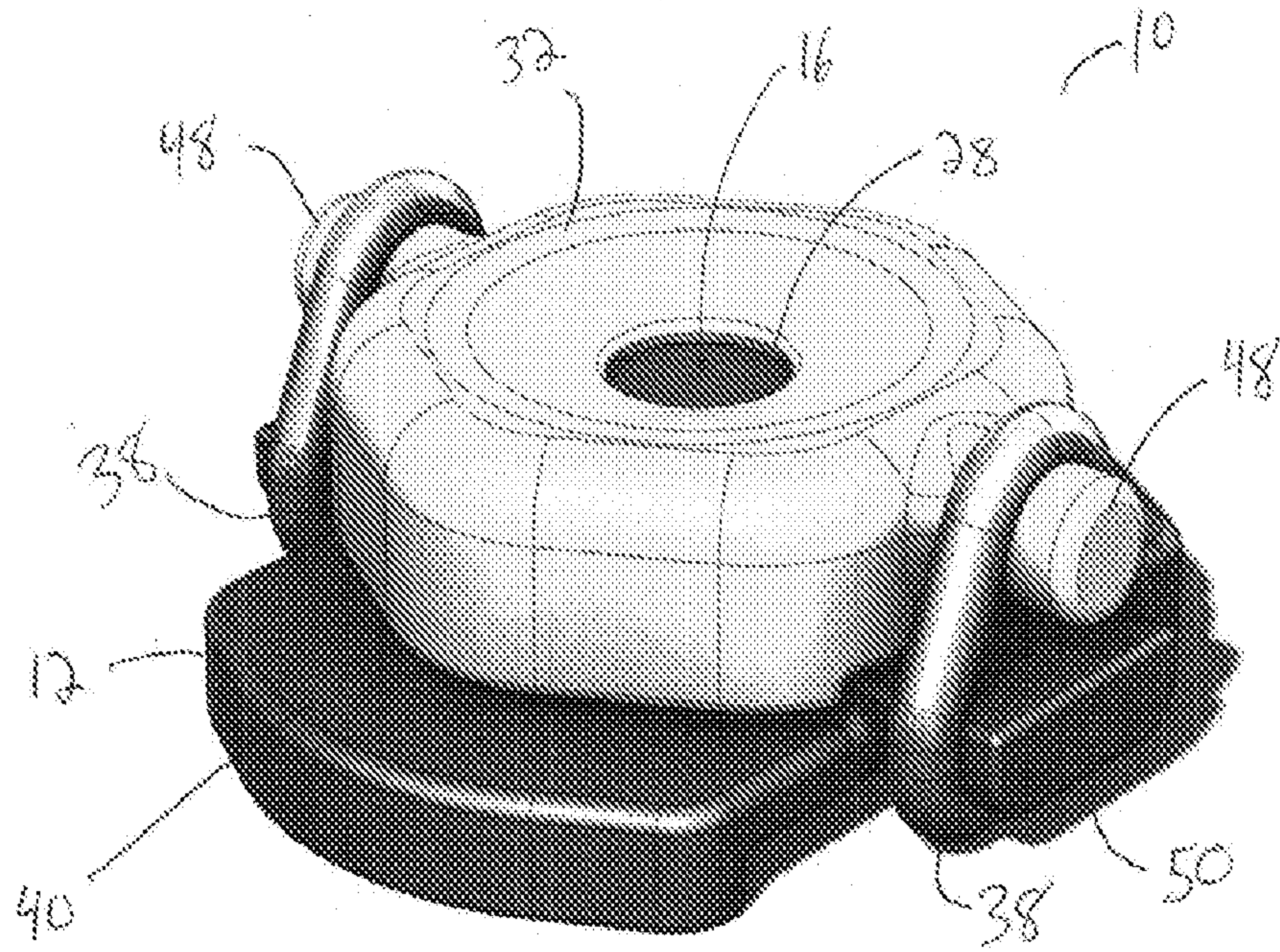


FIG. 10A

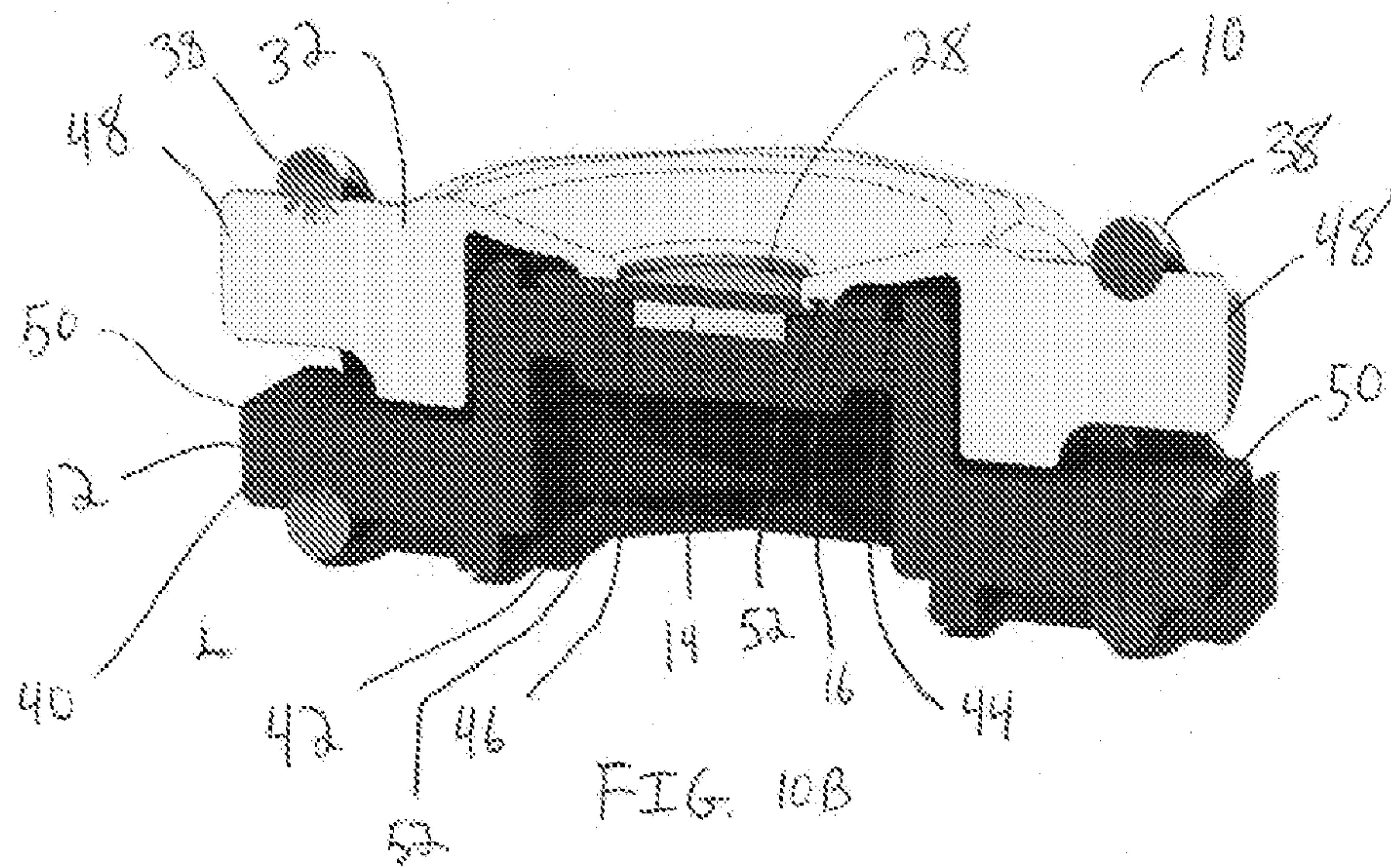
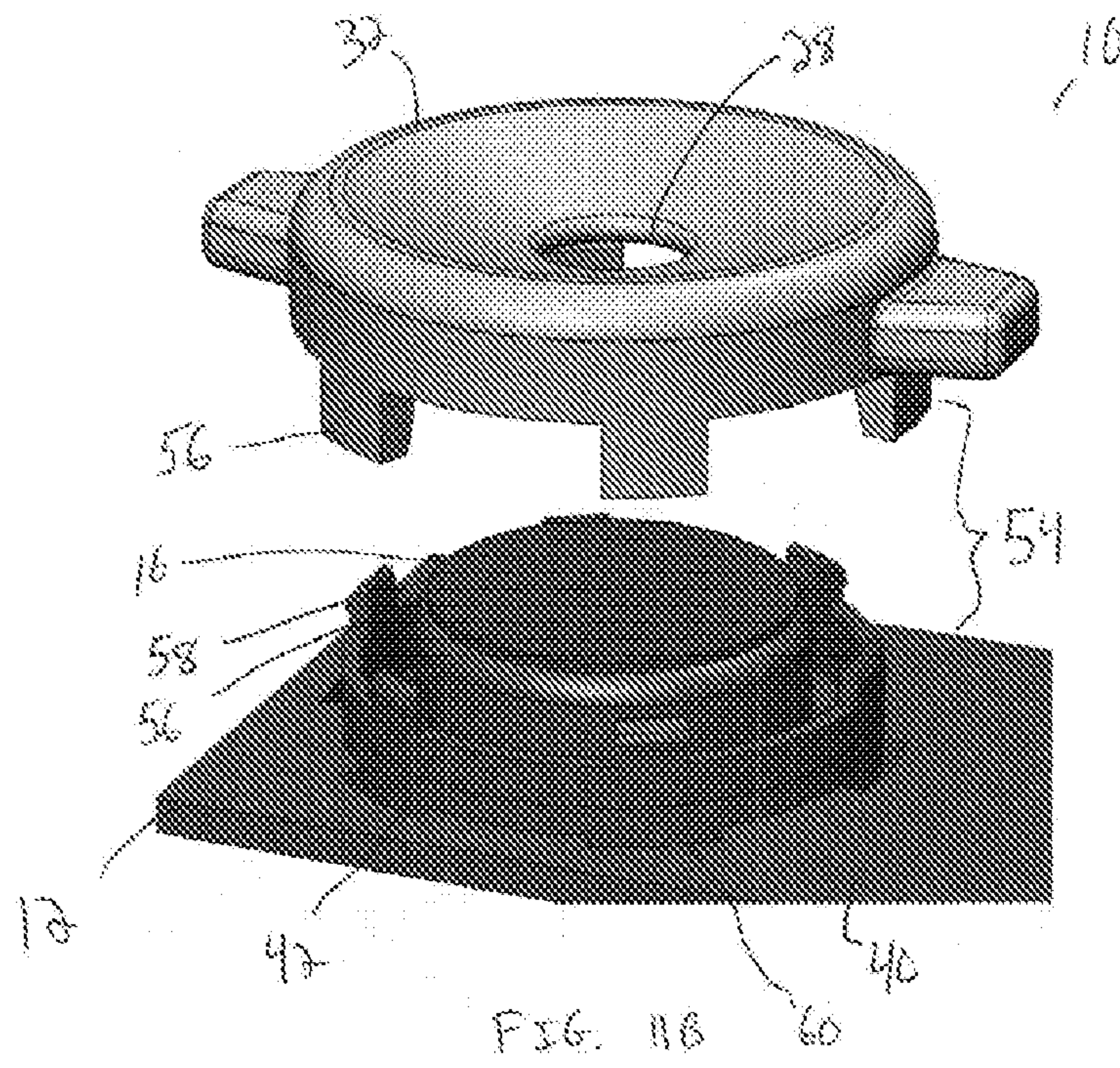
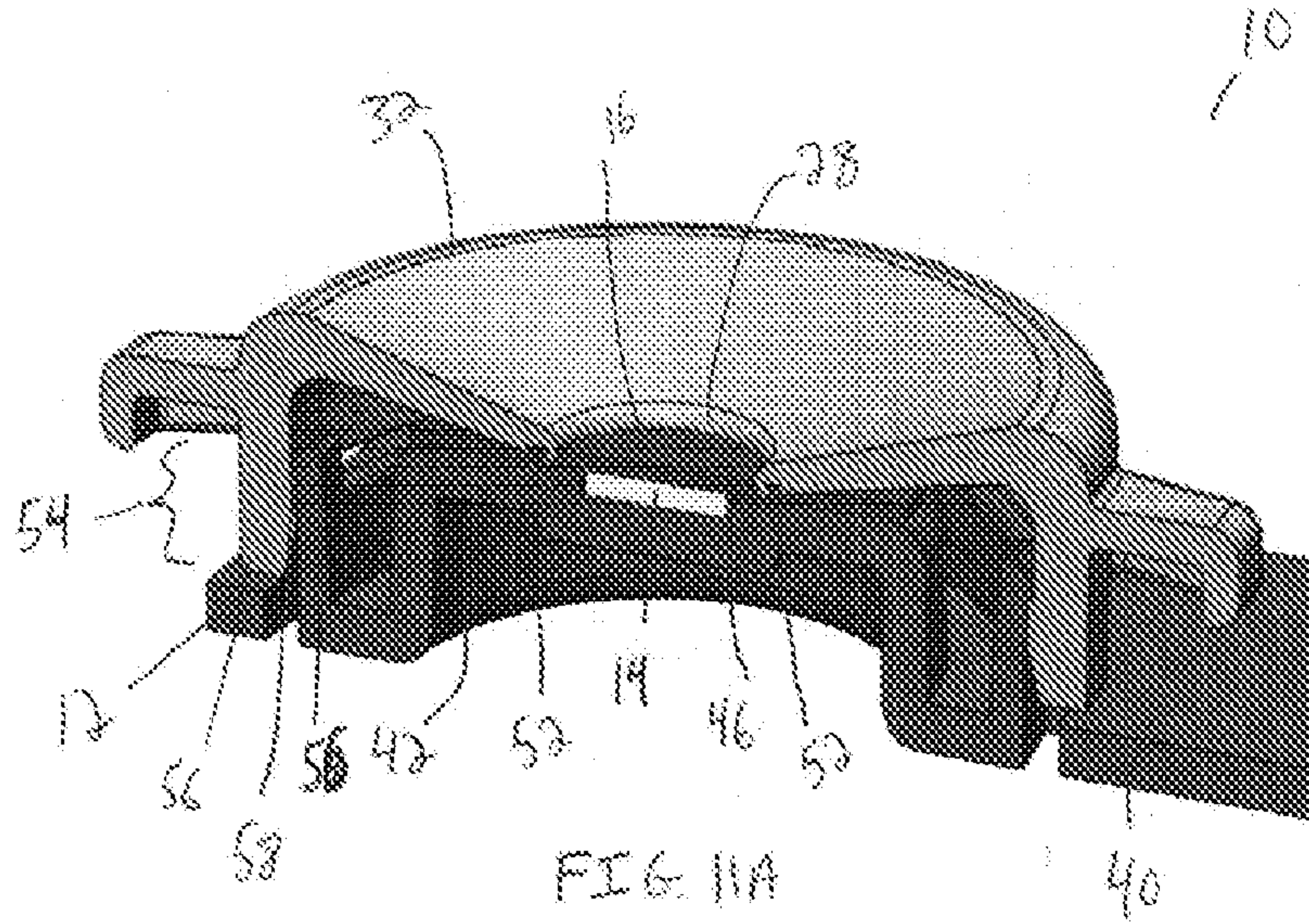


FIG. 10B

14/16



15/16

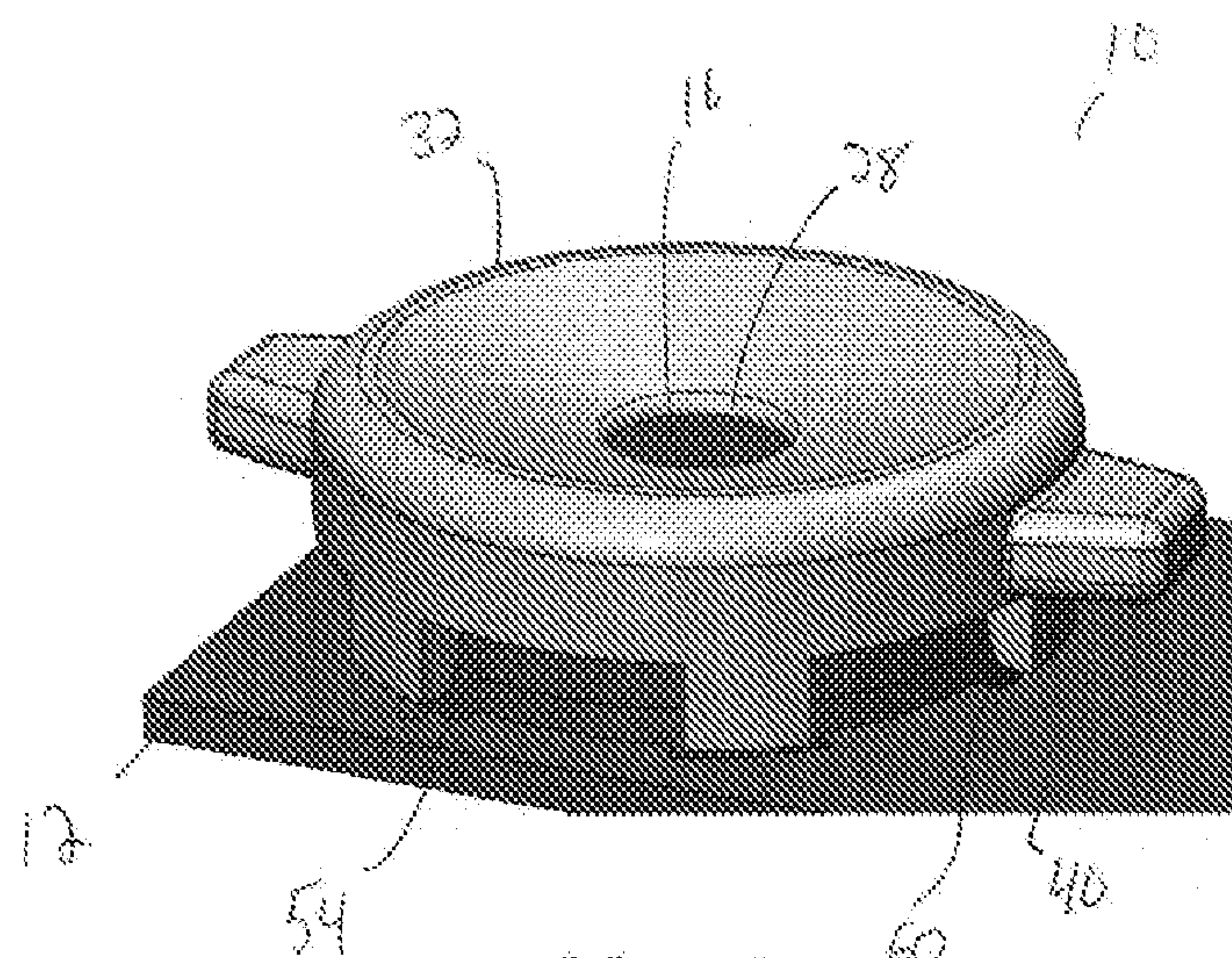


FIG. 11C

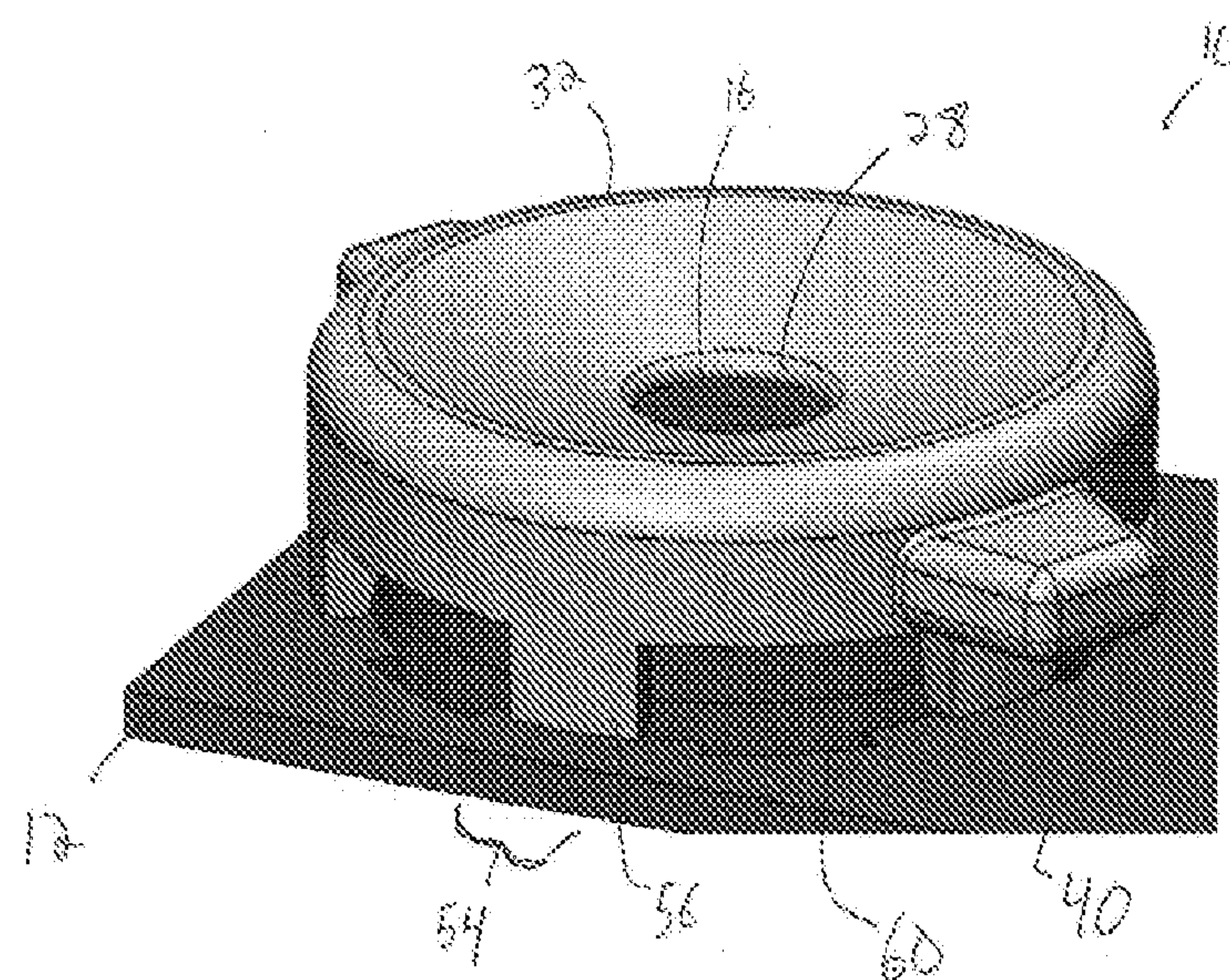
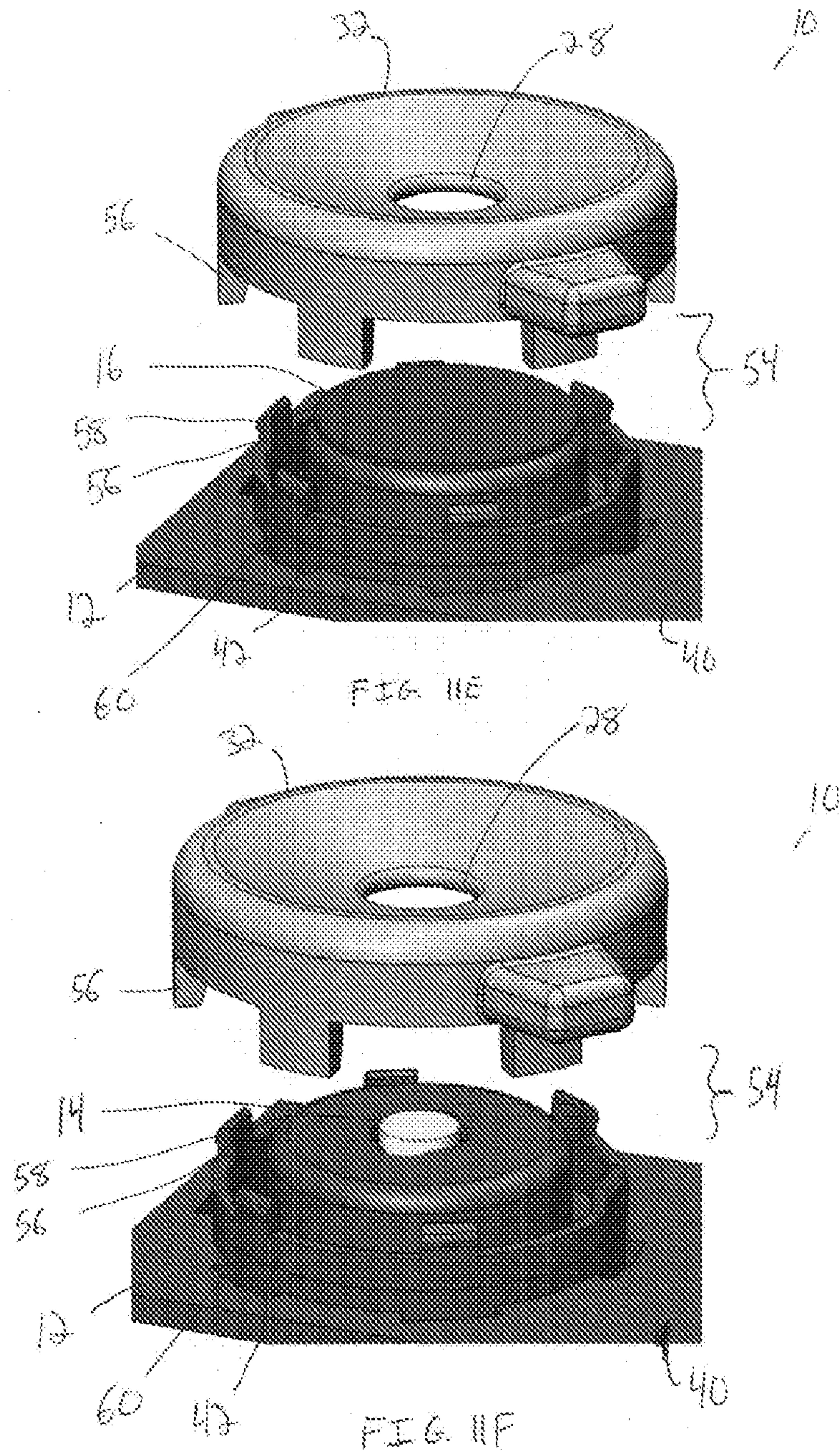


FIG. 11D

16/16



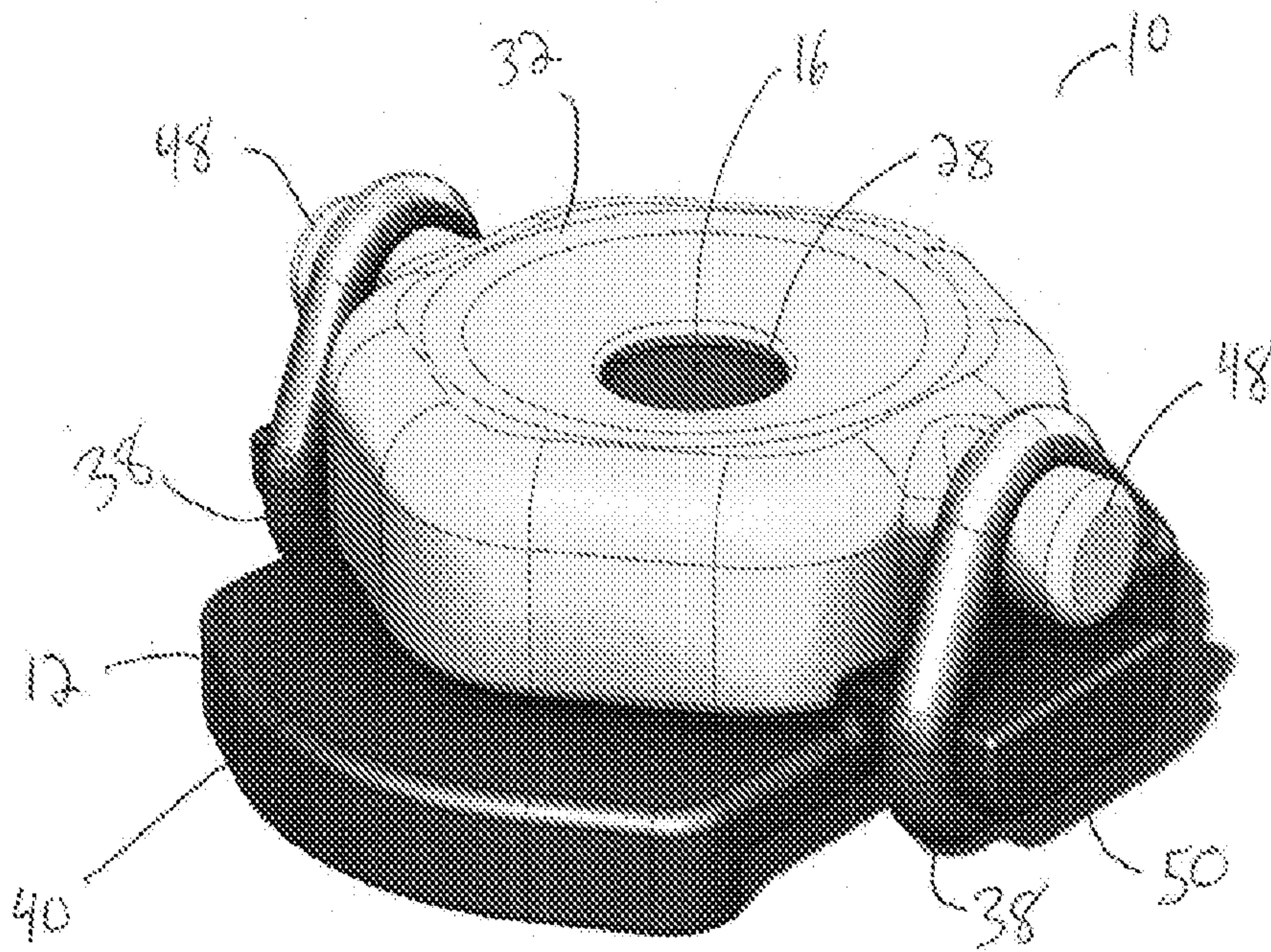


FIG. 10A