Controlling tissue engineered heart valve geometry by using predefined inserts during culture

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(54) Title: CONTROLLING TISSUE ENGINEERED HEART VALVE GEOMETRY BY USING PREDEFINED INSERTS DURING CULTURE

(57) Abstract: Various inserts, called shapers and spacers, are provided for controlling tissue engineered heart valve (TEHV) leaflet geometry during culture. These inserts will prevent TEHV leaflet retraction during culture, be able to control the leaflet geometry during culture, enable culturing TEHV leaflets with a larger coaptation area, control the height of the coaptation area, maintain TEHV leaflet curvatures, and/or enable possibilities to culture TEHV leaflets in open configuration.

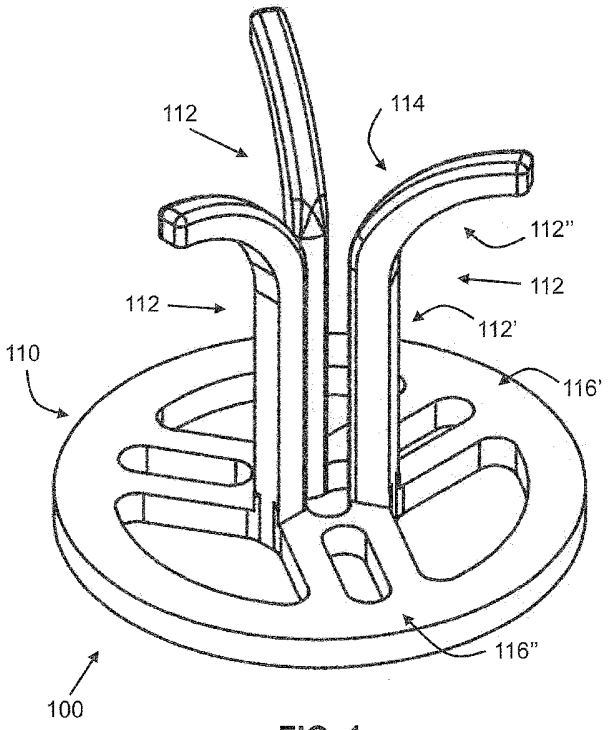

FIG. 1

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CONTROLLING TISSUE ENGINEERED HEART VALVE GEOMETRY BY USING PREDEFINED INSERTS DURING CULTURE

FIELD OF THE INVENTION

This invention relates to devices for tissue engineering. In particular, the invention relates to devices for tissue engineering heart valves.

BACKGROUND OF THE INVENTION

Tissue engineered heart valves (TEHV) are produced by seeding cells on a heart valve shaped scaffold material, followed by a culturing period in a bioreactor system. During culture, the cells will produce an extracellular matrix (ECM).

So far no solutions are available to control heart valve geometry during culture. Contractile cells that are being used will compact the new-formed tissue in all possible directions of constrain. Any predefined scaffold geometry at the start of culture will therefore be lost during culture, resulting in an entirely different geometry after culture compared to the imposed starter geometry.

There are two ways of culturing the TEHV. The first method is to culture the TEHVs in a so-called "open configuration". This means that the individual

heart valve leaflets are separated from each other during culture. The benefit of this approach is that the TEHV leaflets do not have to be separated after culture. The problem with this approach is that because cells will build up tension during culture, they will retract the leaflets, which results in shorted leaflets. In addition, because of the internal tension that builds up in the leaflets, the initially curved shape of the scaffold may be straightened thereby compromising the desired curvature of the leaflets and functionality of the valve.

The second method is to culture the TEHV in a "closed configuration". This means that the valve leaflets are attached to each other, which prevents shortening of the leaflets due to the internal tension that builds up in the leaflets during culture. However, it does not prevent 'straightening' or 'flattening' of the leaflets. In addition, it has been proven to be difficult to achieve a sufficiently large coaptation area between the leaflets in this way, which is crucial for in vivo functionality of the heart valve.

The present invention addresses these problems and provides devices, which allow for the maintenance and control of heart valve geometry during culture.

SUMMARY OF THE INVENTION

The present invention provides devices, methods of using these devices and

systems for controlling tissue engineered heart valve leaflet geometry by using predefined inserts during tissue culture. The inserts are referred to herein as (leaflet) shapers and (leaflet) spacers, which can be used individually or in combination with each other mostly depending on the type of cells cultured with the tissue growth materials and level of geometry shaping/control.

The first insert is a leaflet shaper and has been described herein with several different variations of embodiments. Since we observed that the cells build up tension in all constrained directions, we make use of this effect by inserting a rigid, concave construct that has the shape of the leaflet. The tension that develops in the leaflets will cause the leaflets to compact against the shaper, which acts as a constraint and is capable of controlling the curvature and coaptation of the leaflets.

In some embodiments, the leaflet shaper is covered with small holes to achieve proper nutrient exchange between the medium and the tissue that compacts around the insert. The shaper does not cover the wall of the heart valve such that nutrients and oxygen can be supplied to the wall. Because the tissue compacts against the concave aspect of the shaper, there is no need for a second valve shaped insert/shaper on the other side of the valve leaflets.

The second insert is a leaflet spacer and has been described herein as one embodiment that can be used in combination with the various shapers. When the leaflets are cultured in a closed configuration, the spacer will prevent retraction of the leaflets in the radial direction to constrain the height, and therefore control the size of the coaptation area. It will also enable maintenance of a predefined coaptation area. Hence, this leaflet spacer will constrain the height of the leaflets. A second advantage of the leaflet spacer is to prevent the leaflets from merging over the coaptation area during culture. Since the spacer will be positioned in between the individual leaflets, there is no chance for leaflet concrescence.

An advantage of using the embodiments presented in this invention is that it can result in circumferential collagen orientation in the cultured heart valves, which is beneficial for heart valve functionality.

Another advantage of using the embodiments presented in this invention is that it enables us to culture heart valves without the need of using a complex bioreactor system. In fact, the use of a simple jar would be sufficient. One of the functions of the bioreactor system was to impose the right geometry to the valves by dynamically loading them. But this inserts can achieve the same objective, which is to constrain the imposed geometry.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows according to an exemplary embodiment of the invention a three dimensional view of a shaper **100** for maintaining and controlling the shape of tissue growth material for three leaflets of a heart valve during tissue culture.

FIG. 2 shows according to an exemplary embodiment of the invention a top view of the shaper as shown in **FIG. 1**. The dimensions are in mm.

FIG. 3 shows according to an exemplary embodiment of the invention a bottom view of the shaper as shown in **FIG. 1**. The dimensions are in mm.

FIGs. 4-5 show according to an exemplary embodiment of the invention side views of the shaper as shown in **FIG. 1**. The dimensions are in mm.

FIG. 6 shows according to an exemplary embodiment of the invention a three dimensional view of a shaper **600** for maintaining and controlling the shape of tissue growth material for three leaflets of a heart valve during tissue culture.

FIG. 7 shows according to an exemplary embodiment of the invention a top view of the shaper as shown in **FIG. 6**. The dimensions are in mm.

FIG. 8 shows according to an exemplary embodiment of the invention a bottom view of the shaper as shown in **FIG. 6**. The dimensions are in mm.

FIGs. 9-10 show according to an exemplary embodiment of the invention side views of the shaper as shown in **FIG. 6**. The dimensions are in mm.

FIG. 11 shows according to an exemplary embodiment of the invention a detailed aspect of a meshed surface area with holes from **FIG. 7**. The dimensions are in mm.

FIG. 12 shows according to an exemplary embodiment of the invention a three dimensional view of a shaper **1200** for maintaining and controlling the shape of tissue growth material for three leaflets of a heart valve during tissue culture.

FIG. 13 shows according to an exemplary embodiment of the invention a top view of the shaper as shown in **FIG. 12**. The dimensions are in mm.

FIG. 14 shows according to an exemplary embodiment of the invention a bottom view of the shaper as shown in **FIG. 12**. The dimensions are in mm.

FIGs. 15-16 show according to an exemplary embodiment of the invention side views of the shaper as shown in **FIG. 12**. The dimensions are in mm.

FIG. 17 shows according to an exemplary embodiment of the invention a three dimensional view of a spacer **1700** for further maintaining and controlling the shape of tissue growth material for three leaflets of a heart valve during tissue culture.

FIGs. 18-19 show according to an exemplary embodiment of the invention top views of the spacer as shown in **FIG. 17**. The dimensions are in mm.

FIGs. 20-21 show according to an exemplary embodiment of the invention side views of the spacer as shown in **FIG. 17**. The dimensions are in mm.

FIG. 22 shows according to an exemplary embodiment of the invention the various shapers **100**, **600** and **1200** with spacer **1700**, and tissue growth material **2200** and how they fit and can be used together.

FIG. 23 shows according to an exemplary embodiment of the invention a change from random collagen orientation towards circumferential aligned collagen orientation, due to the leaflet shaper insert during culture.

FIGs. 24-25 show according to exemplary embodiments of the invention *in vitro* results of TEHVs cultured with the use of, for example, but not limited to shaper **1200**.

FIG. 26 shows according to an exemplary embodiment of the invention long term *in vivo* results of TEHVs cultured with the use of, for example, but not limited to shaper **1200**.

DETAILED DESCRIPTION

FIGs. 1-5 show a first embodiment of a shaper **100** for maintaining and controlling heart valve geometry during culture. Shaper **100** is intended for a heart valve with three leaflets and distinguishes a support base **110** and three inner arms **112** each capable of supporting a tissue growth material (not shown) to form one of the leaflets of the heart valve. In this embodiment, it is the mid-axis of the heart valve leaflets that will be constrained and controlled during culture.

Each of the inner arms **112** has a first portion **112'** and a second portion **112''**, which is only indicated for one of the inner arms for clarity purposes. First portion **112'** is disposed normal to support base **110** and disposed proximal to a center of support base **110**. Second portion **112''** is nonlinear and disposed distal to support base **110** and bends away from the center of support base **110**.

The inner arms **112** are distributed in a triangular pattern at support base **110** and are spaced from each other, as is evident in **FIG. 1**, to define enough space to fit at least the respective tissue growth materials. In other words, the

tissue growth materials are placed over and against their respective inner arms **112** at the medial aspects **114** of inner arms **112** (**114** is only indicated for one of the inner arms **112** for clarity purposes). In this embodiment, the respective tissue growth material is extended (not shown) to area **116'** and **116''** forming a wedge-shape growth material and a canopy (e.g. concave) draped over second portion **112''**.

FIGs. 2-5 show an exemplary embodiment of some dimensions of shaper **100**, which are not limited to the invention as a person skilled in the art would readily appreciate that heart valves/leaflets would vary in dimensions and shape. A paper by the same group as the current inventors provides guidelines for some of the dimensions. The paper is entitled "Effects of valve geometry and tissue anisotropy on the radial stretch and coaptation area of tissue-engineered heart valves" by Loerakker et al. and published in Journal of Biomechanics 46 (2013) 1792–1800.

Depending on the type of cells used with the tissue growth material for shaper **100**, there might be a desire to further control the shape and/or spacing between the tissue growth materials draped against the inner arms **112**. For this purpose, spacer **1700** is designed with three surfaces **1710** distributed/oriented with respect to each other in the same triangular pattern as how inner arms **112** are distributed. Side **1720** of spacer **1700** can be placed towards the top of support base **110** and will then sit at the top of the support

base **100** (see also **FIG. 22**). Surfaces **1710** fit in the space left to fit at least the tissue growth material to separate the tissue growth materials supported by each of the linear portions **112'** of the inner arms **112**. In other words, surfaces **1710** will separate the tissue growth materials.

FIGs. 6-11 show a second embodiment of a shaper **600** for maintaining and controlling heart valve geometry during culture, where shaper **600** could be viewed as an extension of shaper **100** with similar structural components. Shaper **600** is intended for a heart valve with three leaflets and distinguishes a support base **610** and three canopy growth surfaces **620** expanded from the second portions of their respective inner arms **112**. It is noted that only one inner arm **112** is indicated in **FIG. 6** for clarity purposes.

Each canopy growth surface **620** is capable of supporting a tissue growth material (not shown) to form one of the leaflets of the heart valve. The canopy growth surfaces **620** define a concave surface when moving away from the center of support base **610** in outer direction.

The canopy growth surfaces **620** are supported by the respective first portions of the inner arms **112** and a pair of outer arms **612'**, **612''** defined for each of the inner arms. Each of the outer arms **612'**, **612''** have a first portion disposed normal to support base **610** and disposed distal to the center of support base **610**.

In other words, each of the canopy growth surfaces **620** further span to the base of support surface **610** along the radial separation of the respective outer arms **612'**, **612''** and inner arm **112** such that each span is capable of supporting the respective growth material. Differently stated, the combinations of each of the first portions of the inner arms **112** with their respective pair of outer arms **612'**, **612''** define wedge-shape growth surfaces each capable of supporting the respective growth material. As a result the tissue growth material for the heart valve leaflets will be constraint and controlled during culture. Open area **630** (indicated for only one of the leaflet canopy growth surfaces for clarity purposes) is left open as it could enhance tissue formation. Holes **640** are intended to allow for improved exchange of nutrients.

The three canopy growth surfaces **620** are distributed in a triangular pattern at support base **610** and are spaced **650** from each other forming a star design, as is evident from e.g. **FIGs. 6-8** especially looking from the top down. The space is defined to fit at least the respective tissue growth materials. In other words, the tissue growth materials are placed over and against their canopy growth surfaces **620** at the medial aspects of canopy growth surfaces **620**. In this embodiment, the respective tissue growth material is extended forming a wedge-shape growth material and a canopy (e.g. concave) draped over the canopy growth surfaces **620**.

FIGs. 7-11 show an exemplary embodiment of some dimensions of shaper **600**, which are not limited to the invention as a person skilled in the art would readily appreciate that heart valves/leaflets would vary in dimensions and shape. The same paper mentioned *supra* provides guidelines for some of the dimensions.

Depending on the type of cells used with the tissue growth material for shaper **600**, there might be a desire to further control the shape and/or spacing between the tissue growth materials draped against the canopy growth surfaces **620**. For this purpose, spacer **1700** is designed with three surfaces **1710** distributed/oriented with respect to each other in the same triangular pattern as how canopy growth surfaces **620** are distributed. Side **1720** of spacer **1700** can be placed towards the top of support base **610** and will then sit at the top of the support base **610** (see also **FIG. 22**). Surfaces **1710** fit in the space left to fit at least the tissue growth material to separate the tissue growth materials supported by each of the canopy growth surfaces **620**. In other words, surfaces **1710** will separate the tissue growth materials.

FIGs. 12-16 show a third embodiment of a shaper **1200** for maintaining and controlling heart valve geometry during culture, where shaper **1200** could be viewed as an extension of shapers **100** and **600** by having some structural components in common. Shaper **1200** is intended for a heart valve with three

leaflets and distinguishes a support base **610** and three canopy growth surfaces **620** expanded from their respective inner arms **112**. It is noted that only one inner arm **112** is indicated in **FIG. 12** for clarity purposes.

Shapers **600** and **1200** are similar with the difference that for shaper **1200** each of the canopy growth surfaces **620** further span to the base of support surface **610** with meshes surfaces **1210** between the respective outer arms **612'**, **612"** and inner arm **112**. Only one of the meshed surfaces is indicated for clarity purposes. It is also noted that a wedge shaped surface forms the basis for each of the concave parts of the canopy growth surfaces.

Another difference is that the meshes surface **1210** have holes, like holes **640**, to allow exchange of nutrients. Each of these canopy growth surfaces **620** is capable of supporting the respective growth material. Similar to shaper **600**, spacer **1700** can be used for shaper **1200** to fit in the space **650** left to fit at least the tissue growth material to separate the tissue growth materials supported by the meshed surfaces.

FIGs. 13-16 show an exemplary embodiment of some dimensions of shaper **1200**, which are not limited to the invention as a person skilled in the art would readily appreciate that heart valves/leaflets would vary in dimensions and shape. The same paper mentioned *supra* provides guidelines for some of the dimensions.

In summary, **FIG. 22** shows the various shapers **100**, **600** and **1200** with spacer **1700**, and tissue growth material **2200** and how they fit and can be used together. There are various variations one could imagine, such as that these embodiments can be constructed for a single-leaflet heart valve, bi-leaflet (two leaflet) heart valve or multiple-leaflet heart valve. The design principles for these different heart valves would be similar to the tri-leaflet heart valve with the difference of the number of inner arms for shaper **100**, the number of canopy growth surfaces for shaper **600** and **1200**, the shape of the space with the growth surfaces and various others as a person skilled in the art would readily appreciate. In addition, dimensions (including the radius/angles of the canopy growth surfaces) shown in the exemplary embodiments could be varied to fit the desired objective for the tissue engineered heart valves.

The manufacturing of the inserts could be via conventional computer numerical control (CNC) milling technology with biocompatible materials such as polyether ether ketone (PEEK) or via rapid prototyping techniques like three-dimensional printing with materials such as acrylonitrile butadiene styrene (ABS) or more biocompatible materials such as PLA. However, other conventional manufacturing techniques would still suffice. In addition, the shapers and spacers could be made as modular components that could be assembled to for example come up for a single-leaflet, bi-leaflet or tri-leaflet design.

Circumferential collagen alignment

Circumferential collagen alignment in TEHVs will result in radial leaflet stretch while being hemodynamically loaded, which is beneficial for the opening and closing behavior of the valve. As shown in **FIG. 23**, the starter matrix of the TEHV contains mainly randomly organized scaffold fibers. When the cells are seeded onto the construct, they will start producing randomly organized collagen matrix along these scaffold fibers. During culture, the scaffold material will hydrolyze and lose mechanical functionality. From this point on cells will start pulling in the direction of constrained. Since the leaflet shaper insert is a rigid body, cells will compact around this insert and realign the collagen in the direction of constrain. This will result in circumferentially aligned collagen orientation (**FIG. 23**).

Static Valve Culture

Currently TEHVs are being cultured in a sophisticated bioreactor system. This system is regulating pulsatile pressures onto the leaflets in combination with regulated medium flow to enhance tissue formation. We found out that by using the insert as presented herein during culture, the bioreactor system can be replaced by a simple jar. Since the insert is required to maintain the initial heart valve geometry, it is hampering the pulsatile pressures exerted on the leaflets, which makes the main function of the bioreactor system redundant or obsolete. It seems that when the fluid flow is maintained, it would still be

possible to culture functional TEHVs. This finding can have a big impact in the way TEHVs can be produced in a future commercial way. Without the use of a complicated bioreactor system, valve production can be up scaled easily and will lower the production costs.

Results

FIGs. 24-25 show examples of *in vitro* results of TEHVs cultured with the use of for example shaper **1200**. After removal of shaper **1200** the TEHV maintained the imposed geometry. **FIG. 24** shows results for a closed configuration with no leaflet retraction **2410**, maintenance of leaflet curvature **2420** and a controlled coaptation area **2430**. **FIG. 25** shows results for an open configuration with leaflets shaped around the shaper insert **2510**, maintenance of leaflet curvature **2520** and a controlled coaptation area **2530**.

FIG. 26 shows an example of long term *in vivo* results of TEHVs cultured with the use of for example shaper **1200**. Up to 24 weeks, the heart valve maintained its initial geometry and showed no signs of leaflet retraction. These results confirm that the initial geometry of the heart valve after culture is decisive for the final long-term outcome, which can only be obtained by using the leaflet shaper insert during culture.

CLAIMS

What is claimed is:

1. A heart valve cell culturing device, wherein the heart valve comprises at least two leaflets, comprising:

(a) a support base; and

(b) at least two inner arms each capable of supporting a tissue growth material to form one of the leaflets,

wherein each of the inner arms has a first portion and a second portion,

wherein the first portion is disposed normal to the support base and disposed proximal to a center of the support base,

wherein the second portion is nonlinear and disposed distal to the support base and bends away from the center of the support base,

wherein the at least two inner arms are distributed in a pattern at the support base, and

wherein the at least two inner arms are spaced from each other defining enough space to fit at least the respective tissue growth materials.

2. The heart valve cell culturing device as set forth in claim 1,

wherein each of the inner arms further comprises a canopy growth surface expanded from the second portion of the respective inner arms,

wherein each of the canopy growth surfaces define a concave surface when moving away from the center of the support base in outer direction.

wherein each of the canopy growth surfaces is supported by the first portions of the inner arms and a pair of outer arms defined for each of the inner arms, wherein each of the outer arms have a first portion disposed normal to the support base and disposed distal to the center of the support base, and

wherein each of the canopy growth surfaces are capable of supporting the respective growth materials.

3. The heart valve cell culturing device as set forth in claim 2, wherein the canopy growth surfaces comprise holes to allow exchange of nutrients.

4. The heart valve cell culturing device as set forth in claim 2, wherein each of the canopy growth surfaces further span to the base of the support surface along the radial separation of the respective outer arms and inner arm, wherein each span capable of supporting the respective growth material.

5. The heart valve cell culturing device as set forth in claim 2, wherein each of the canopy growth surfaces further span to the base of the support surface and form meshes surfaces

between the respective outer arms and inner arm each capable of supporting the respective growth material.

6. The heart valve cell culturing device as set forth in claim 5, further comprising a spacer to fit in the space left to fit at least the tissue growth material to separate the tissue growth materials supported by the meshed surfaces.

7. The heart valve cell culturing device as set forth in claim 5, wherein the meshes surfaces comprise holes to allow exchange of nutrients.

8. The heart valve cell culturing device as set forth in claim 2, wherein the combinations of each of the first portions of the inner arms with their respective pair of outer arms define wedge-shape growth surfaces each capable of supporting the respective growth material.

9. The heart valve cell culturing device as set forth in claim 8, wherein the wedge-shape growth surfaces comprise holes to allow exchange of nutrients.

10. The heart valve cell culturing device as set forth in claim 8, further comprising a spacer to fit in the space

left to fit at least the tissue growth material to separate the tissue growth materials supported by the wedge-shape growth surfaces.

11. The heart valve cell culturing device as set forth in claim 1, further comprising a spacer to fit in the space left to fit at least the tissue growth material to separate the tissue growth materials supported by each of the linear portions of the inner arms.

12. The heart valve cell culturing device as set forth in claim 1, wherein the at least two inner arms have three inner arms and the pattern is a triangular pattern.

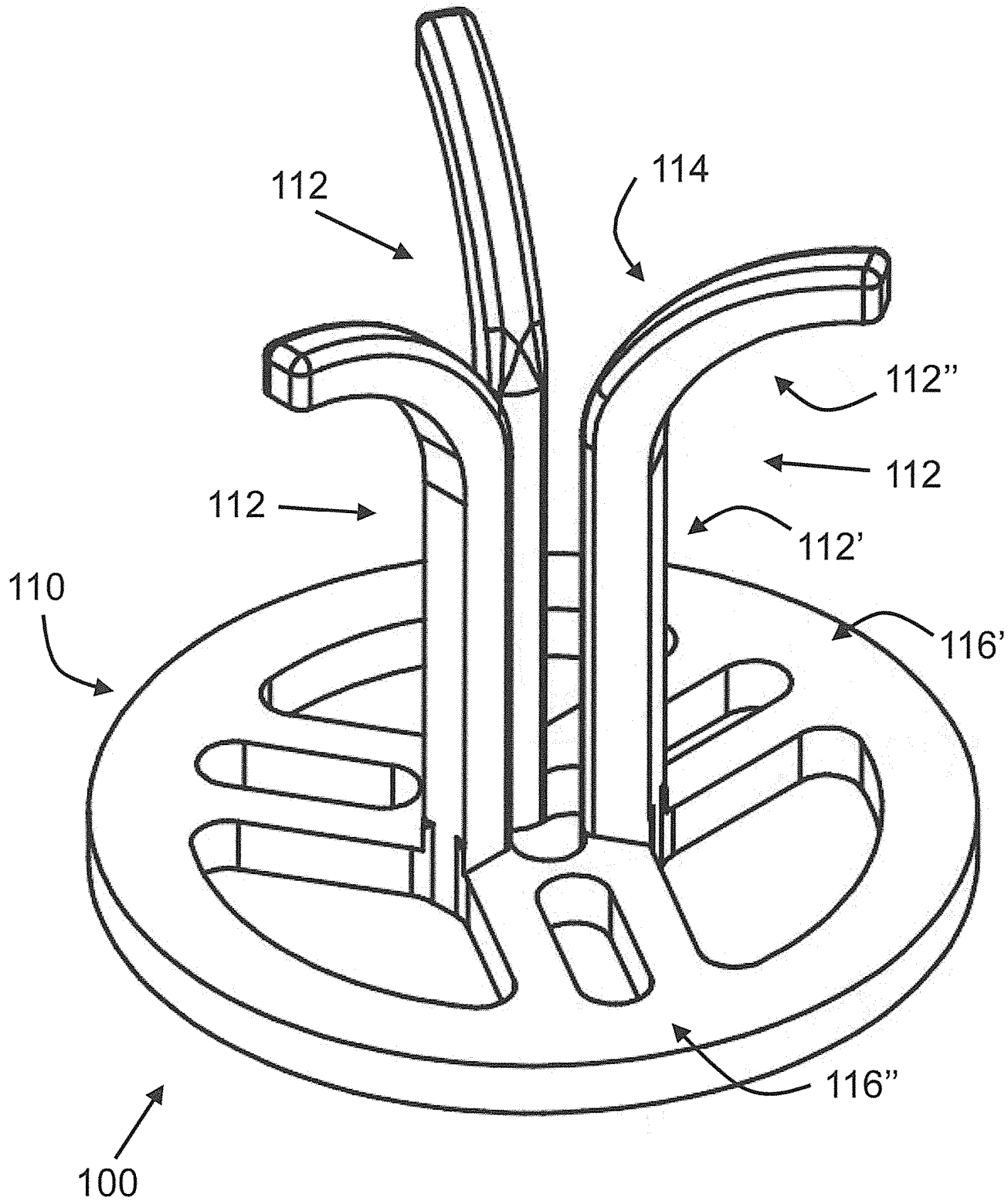

FIG. 1

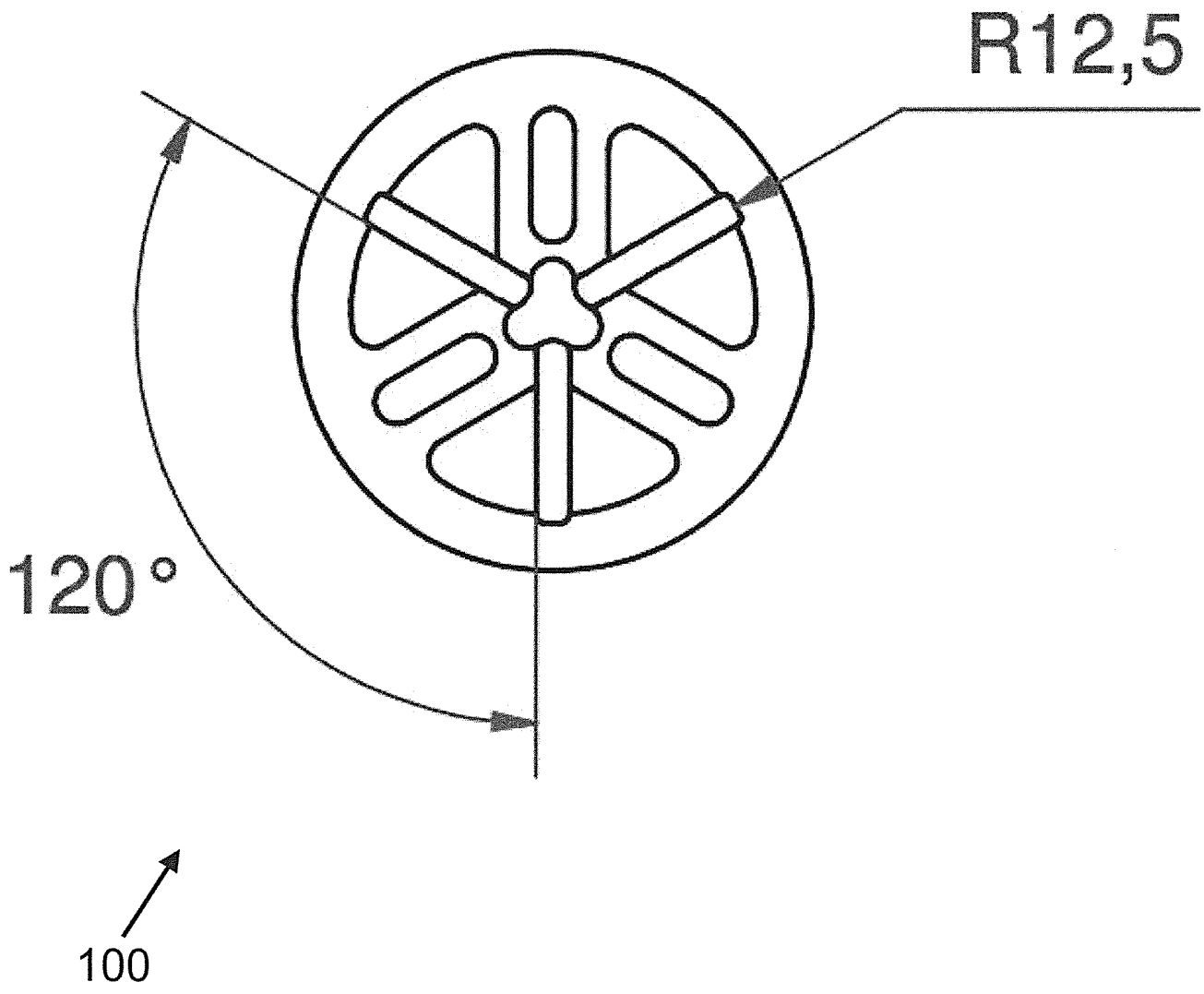

FIG. 2

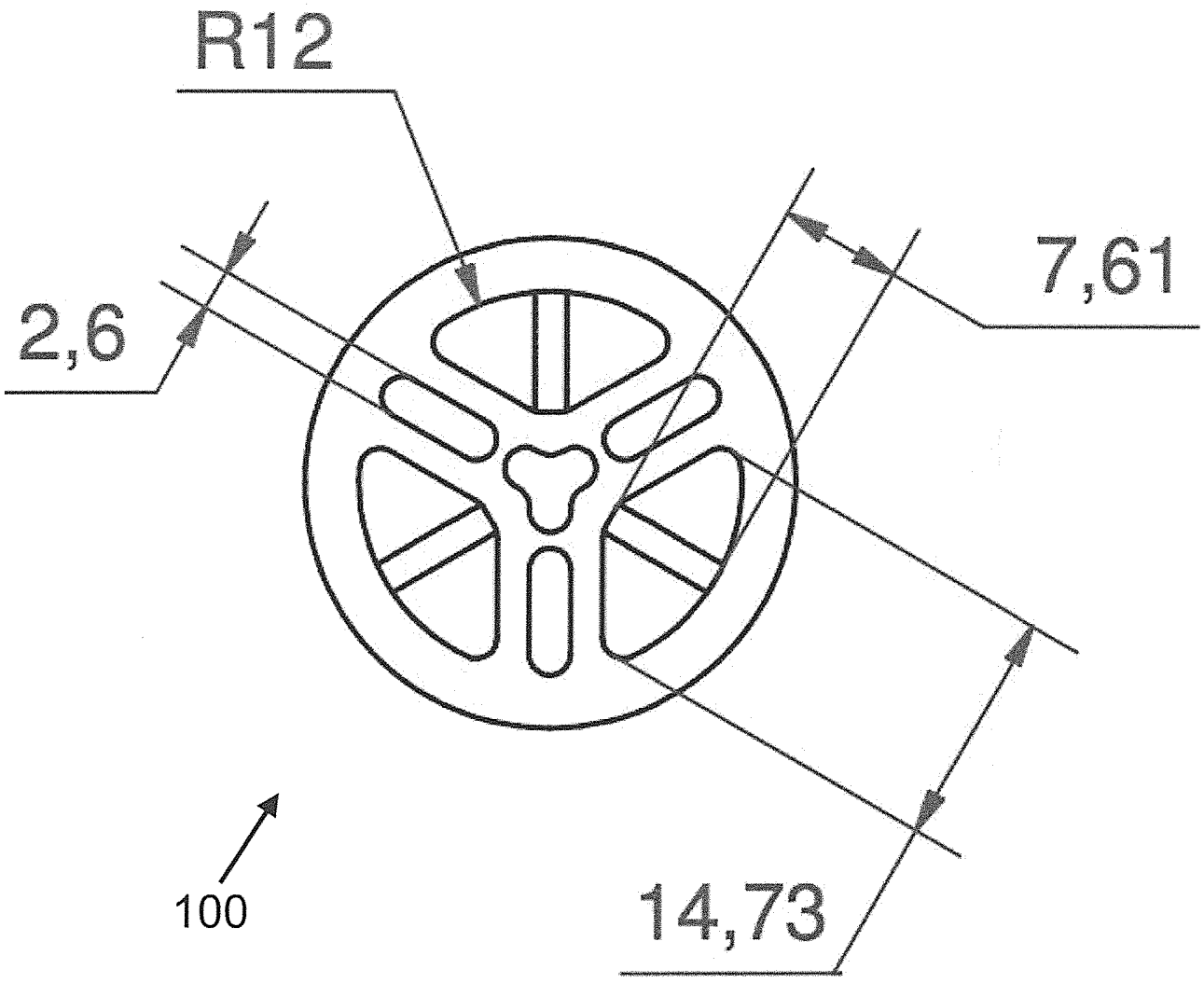

FIG. 3

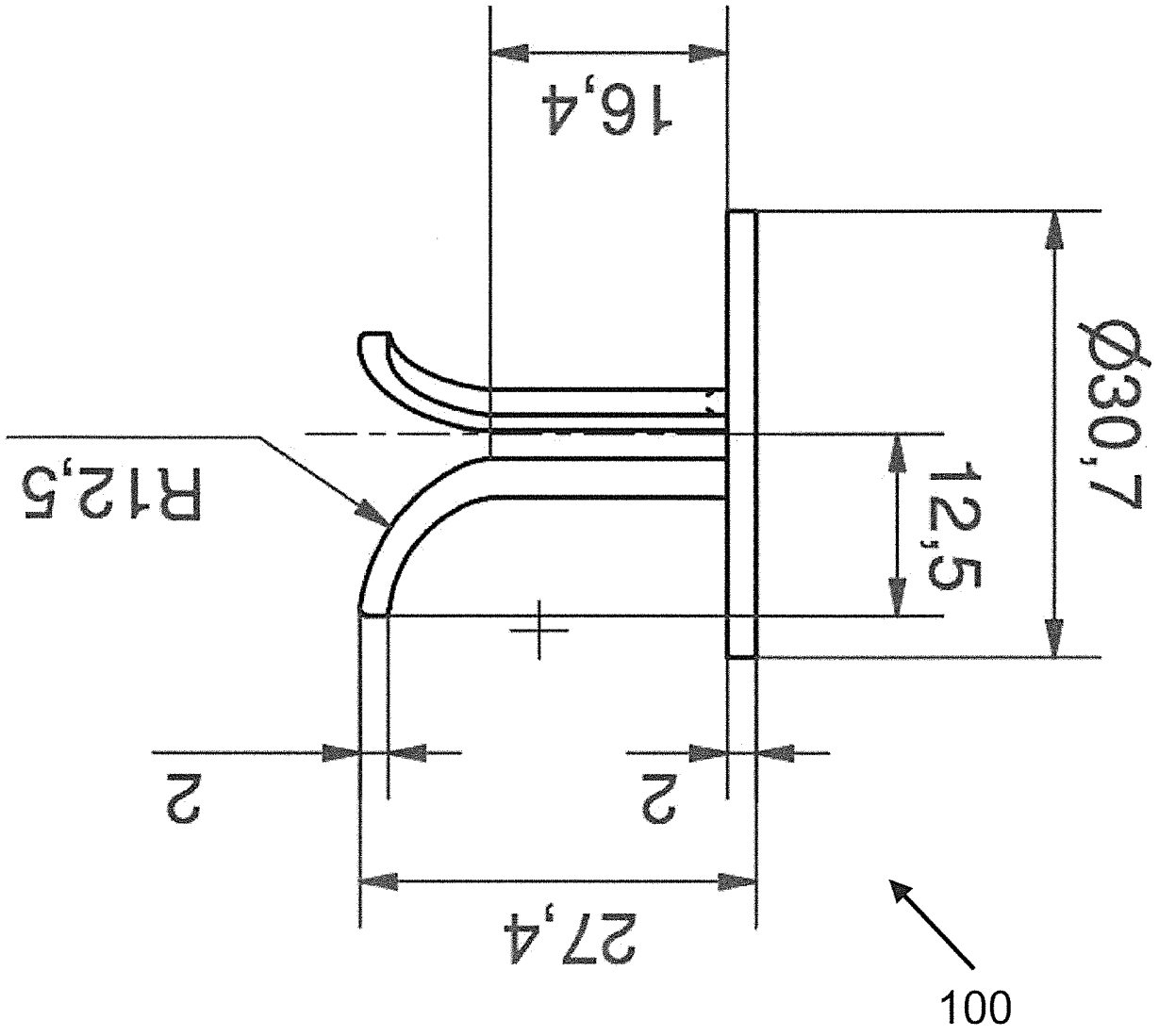

FIG. 4

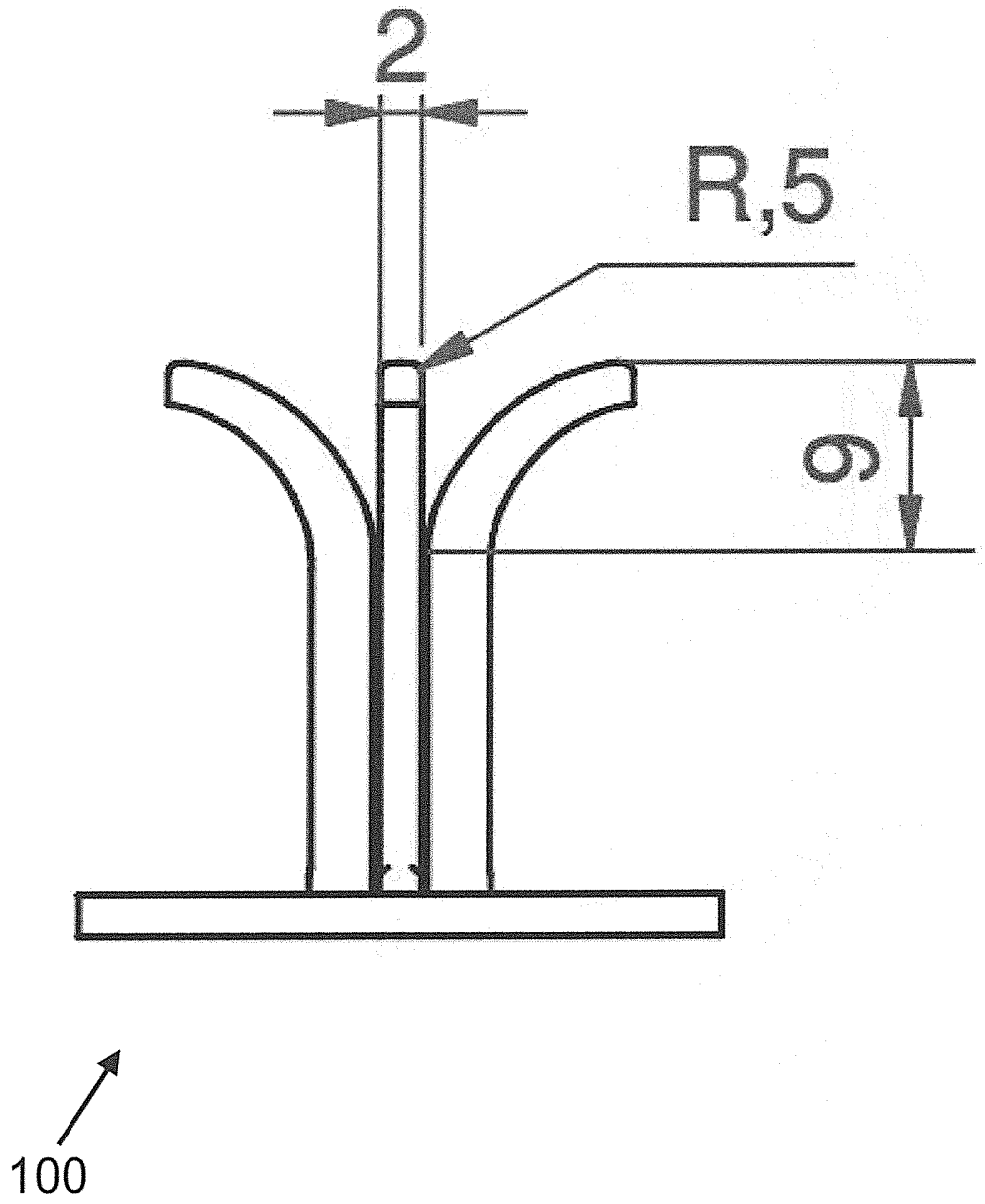

FIG. 5

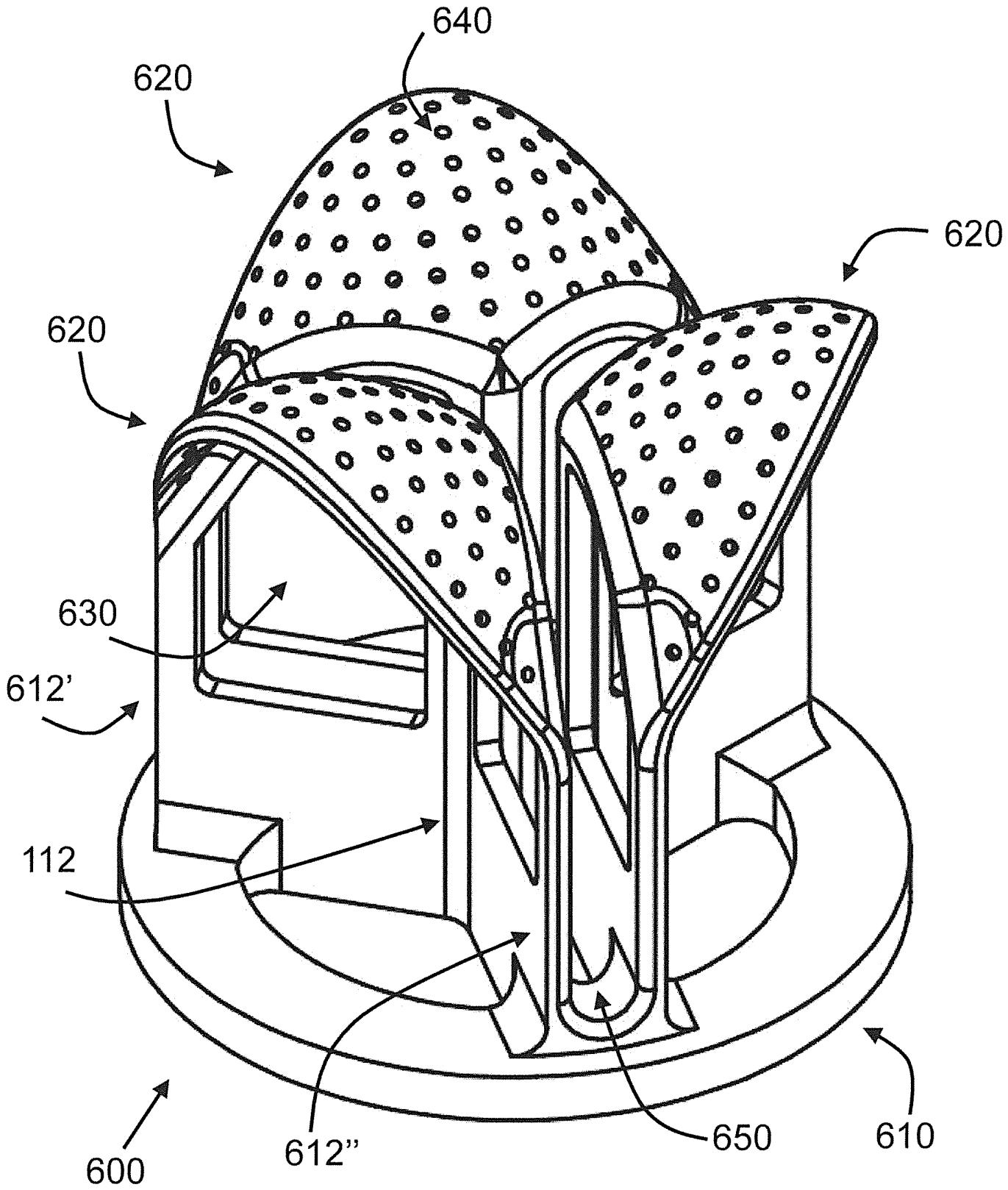

FIG. 6

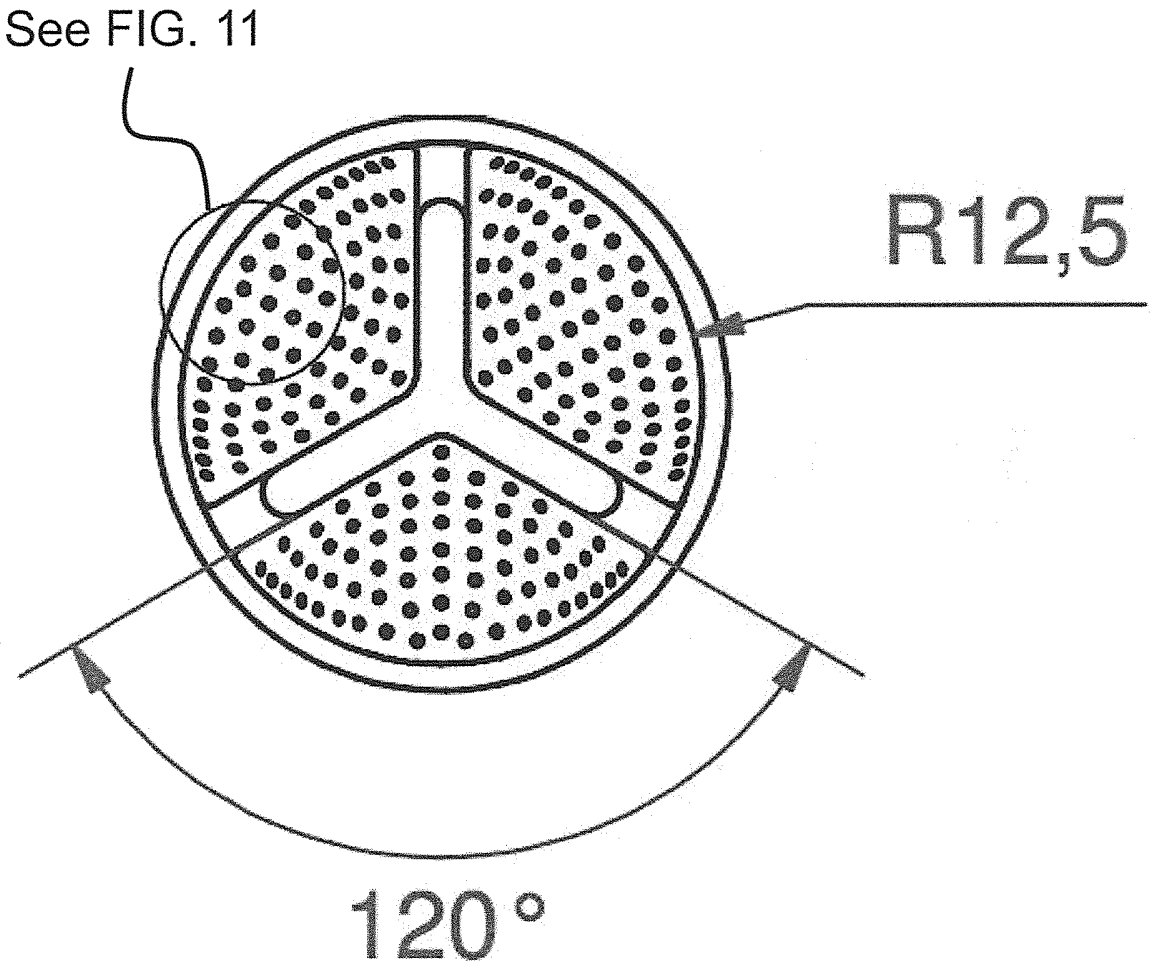

FIG. 7

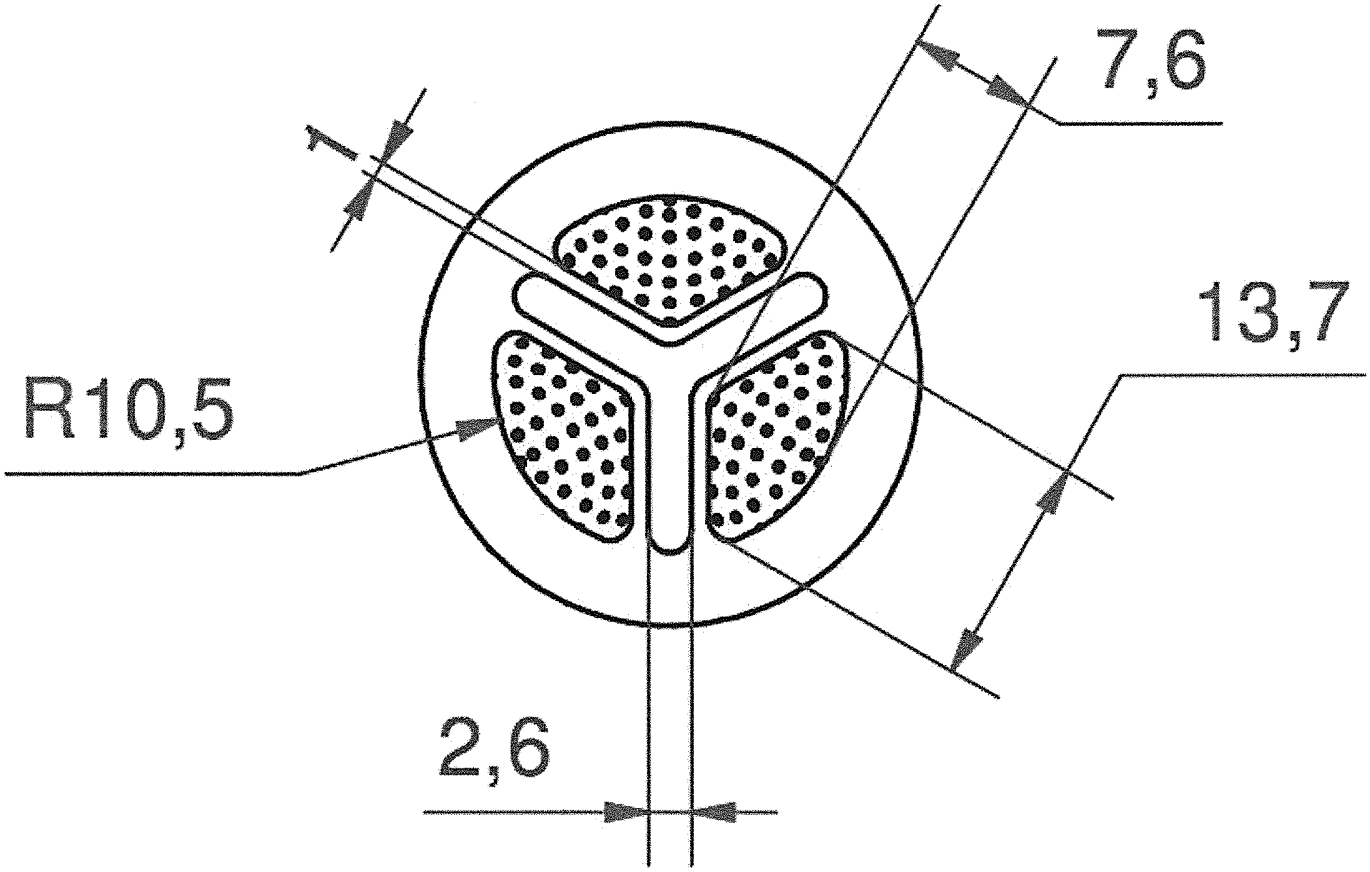

FIG. 8

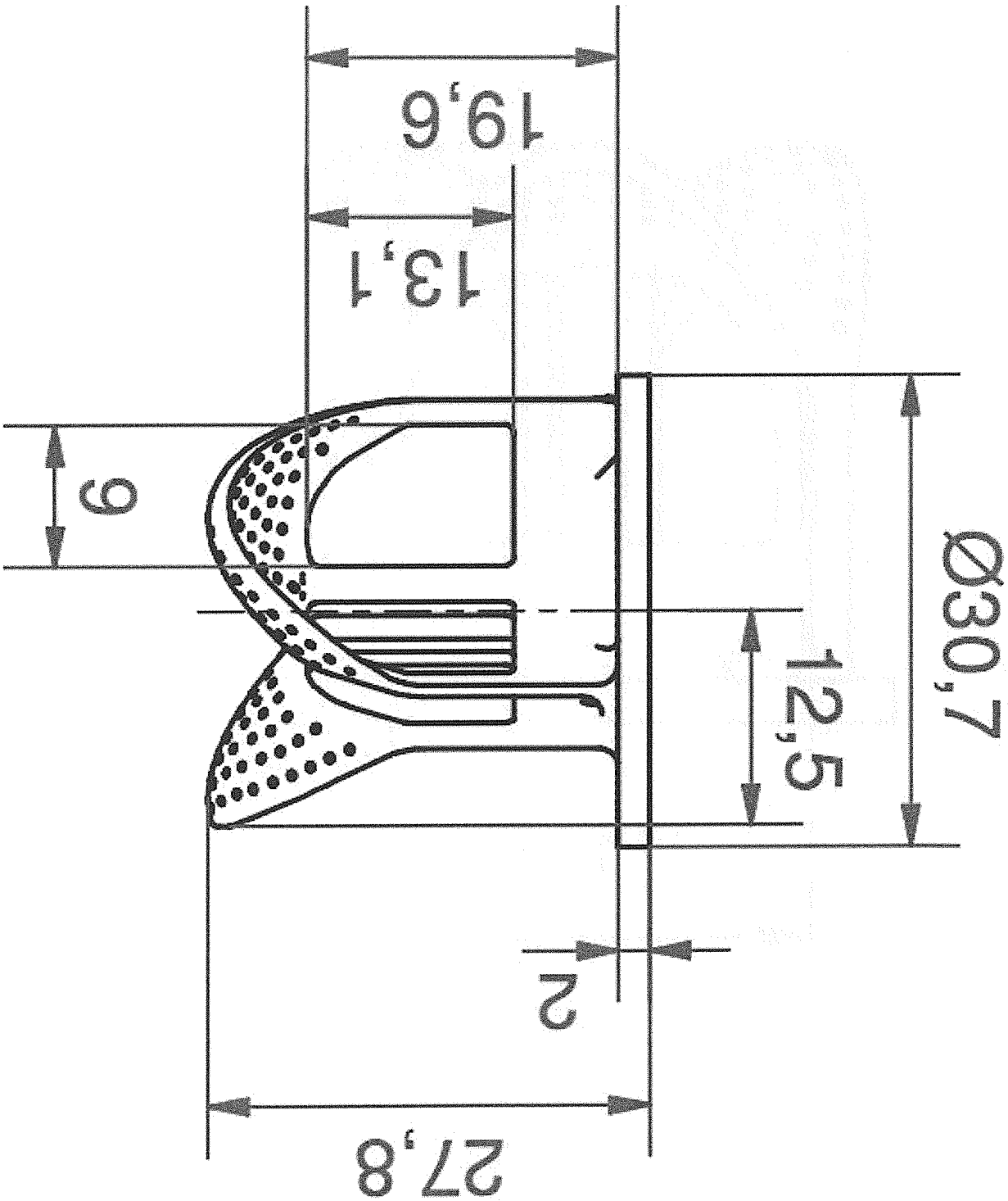

FIG. 9

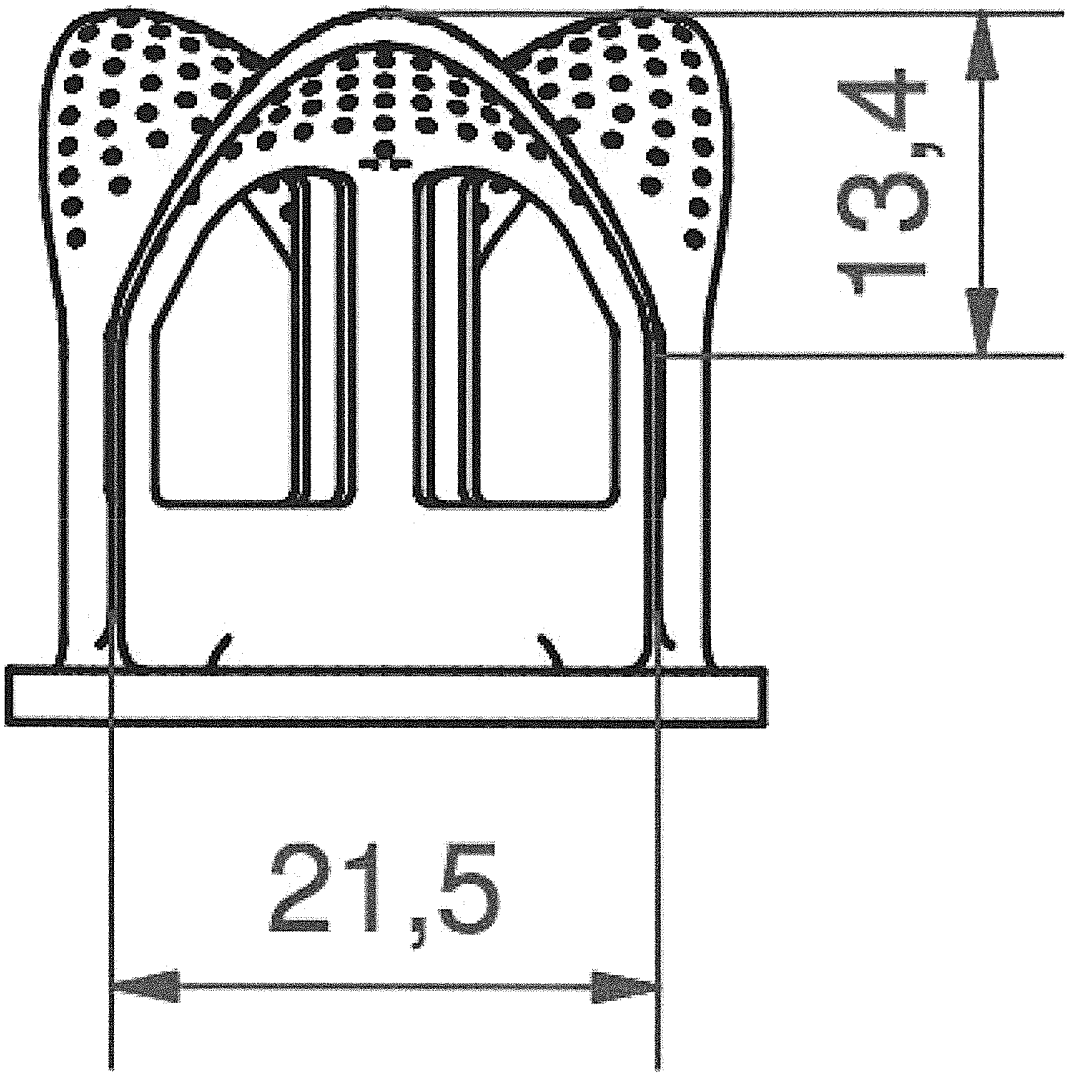

FIG. 10

Aspect from FIG. 7

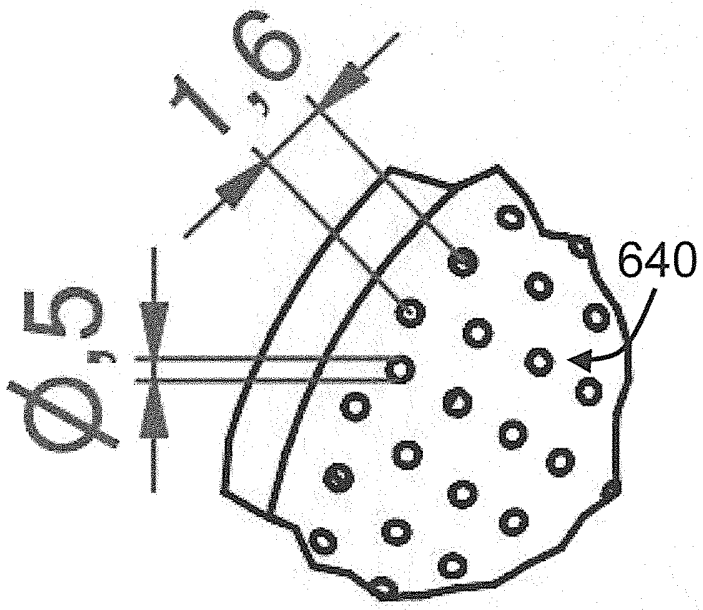

FIG. 11

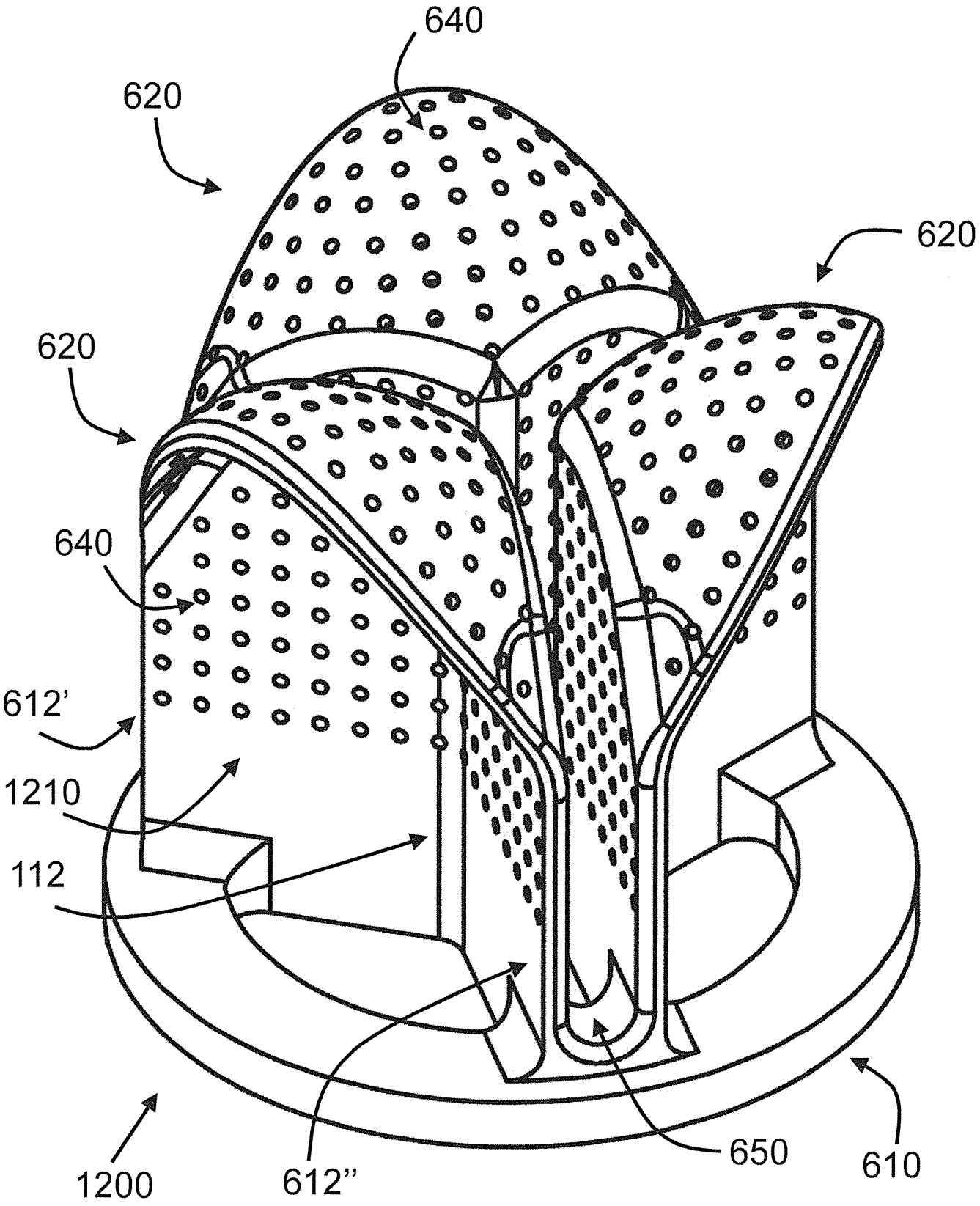

FIG. 12

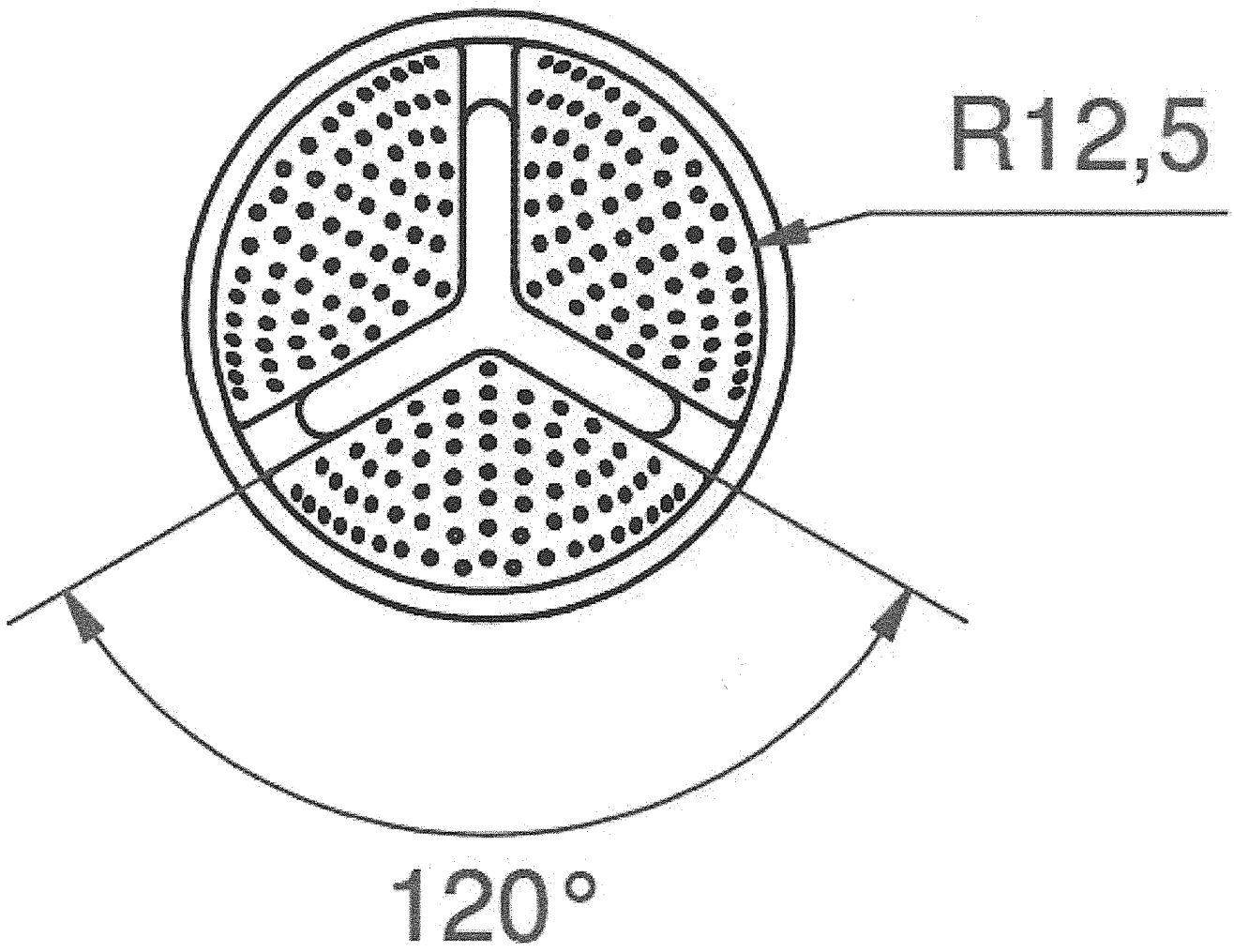

FIG. 13

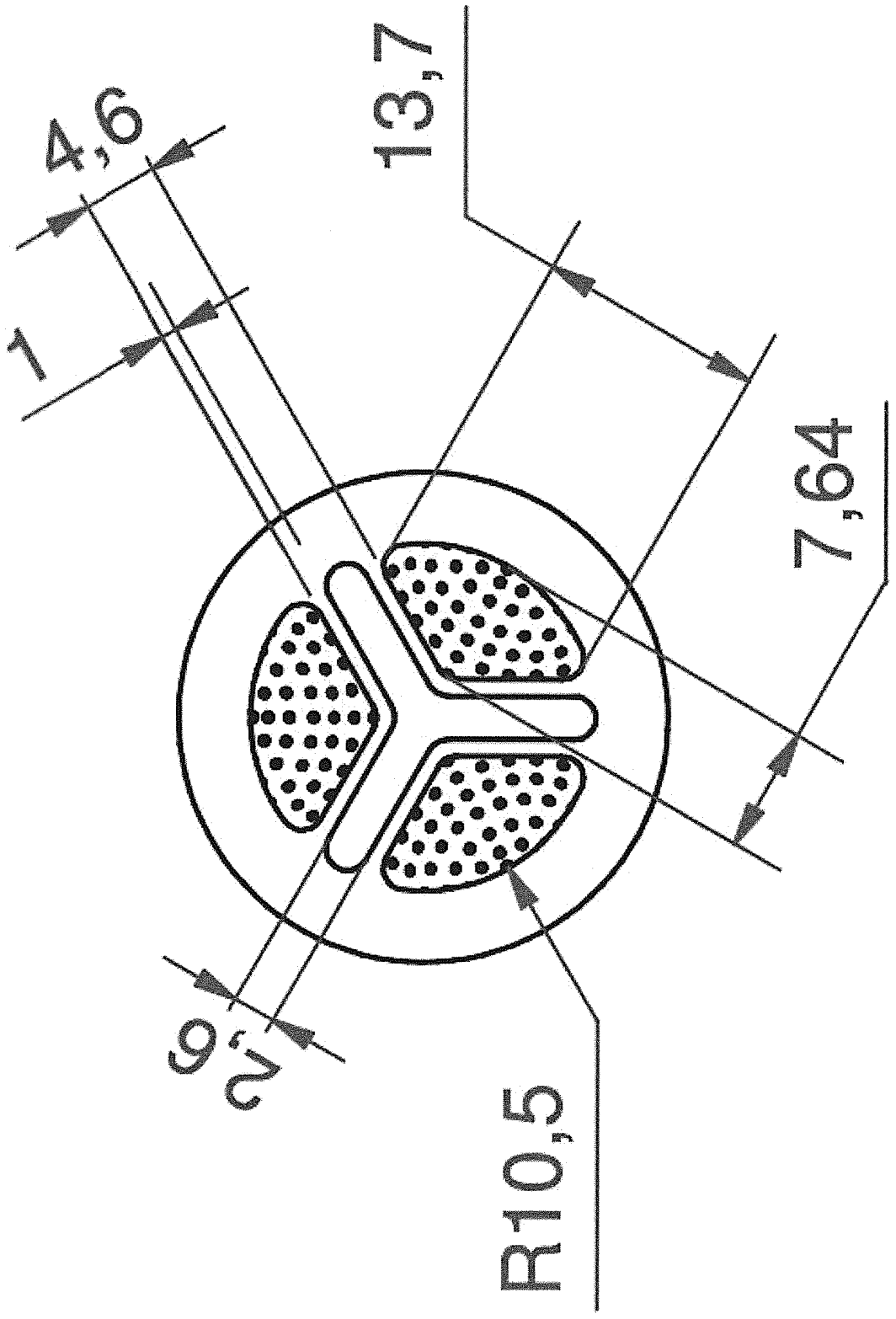

FIG. 14

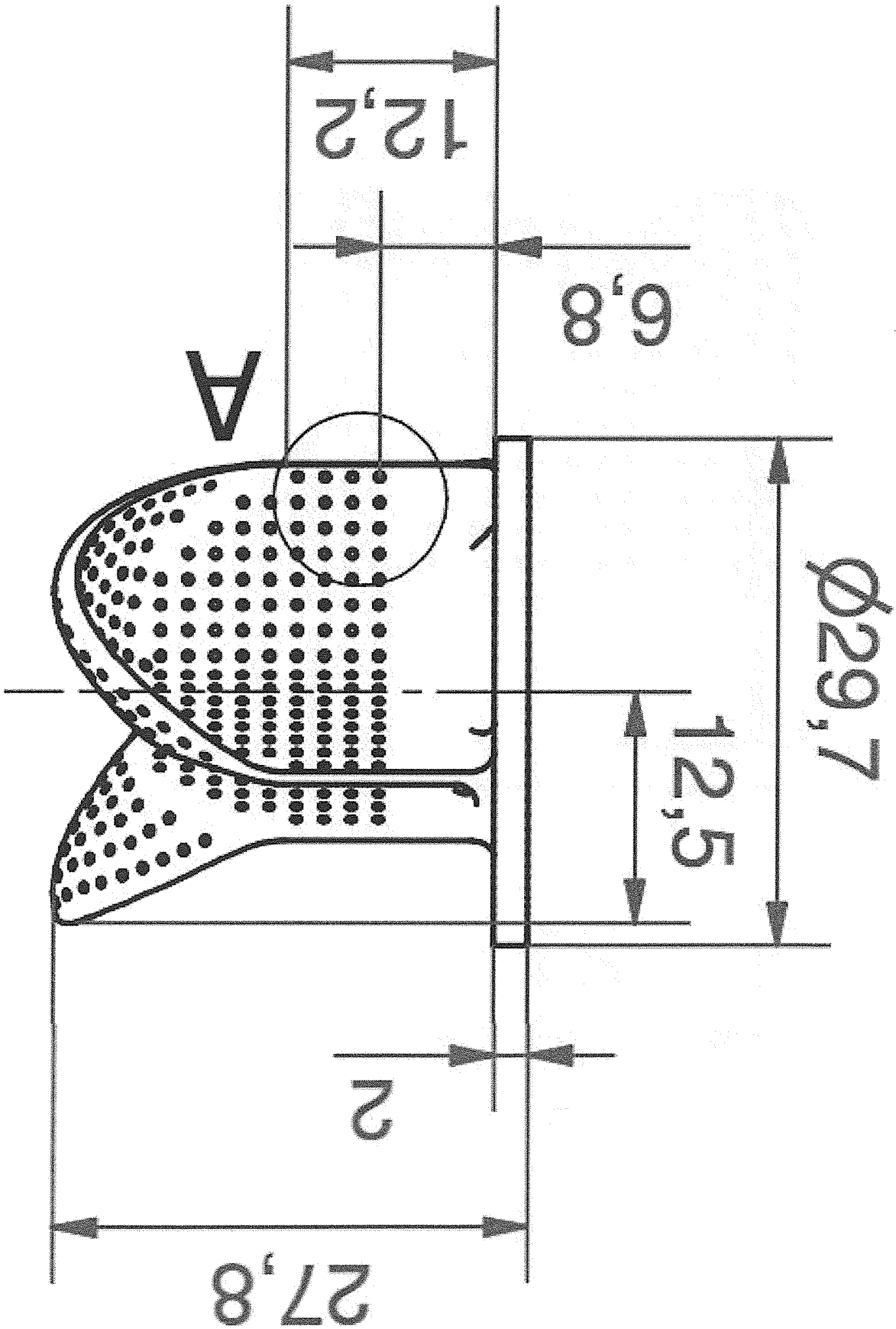

FIG. 15

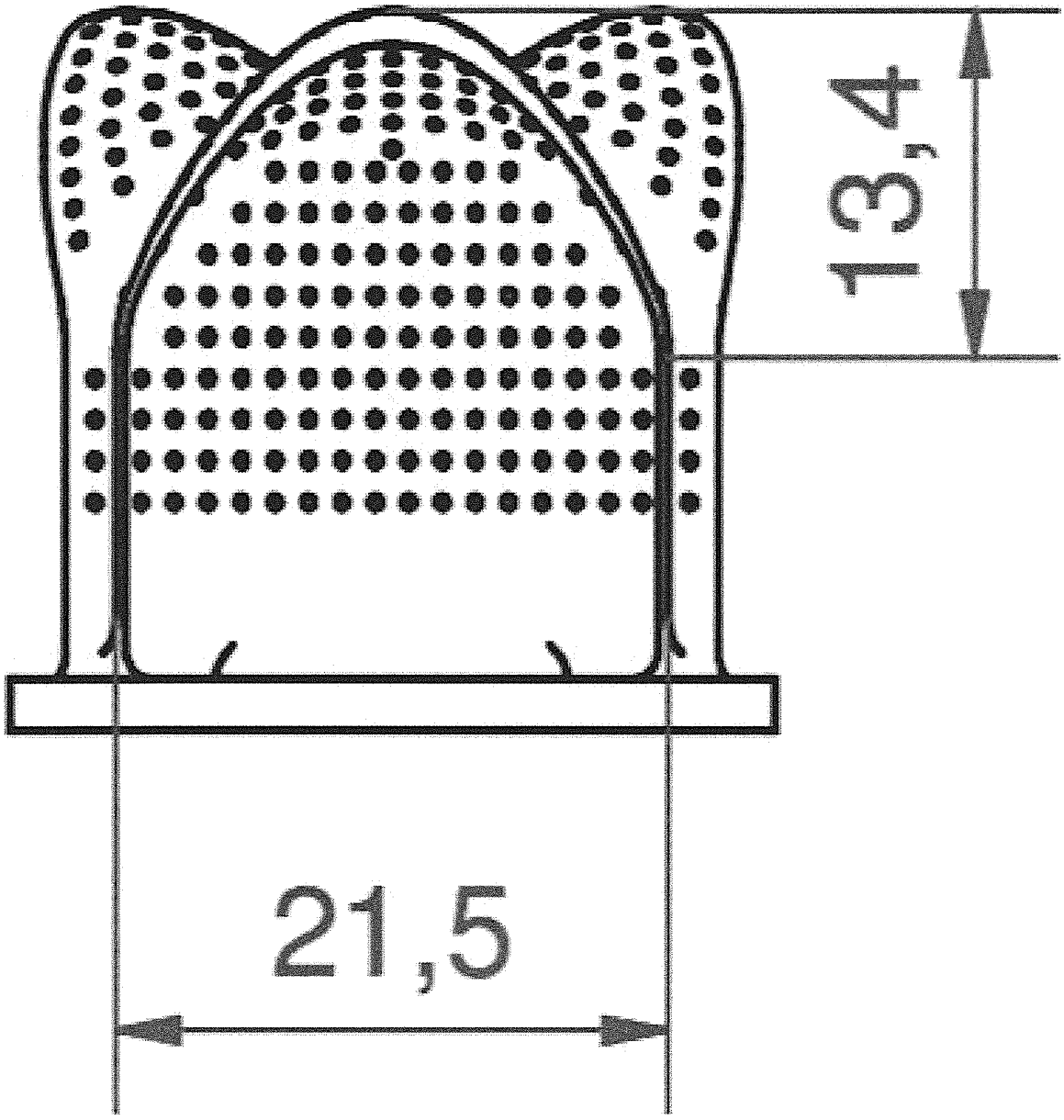

FIG. 16

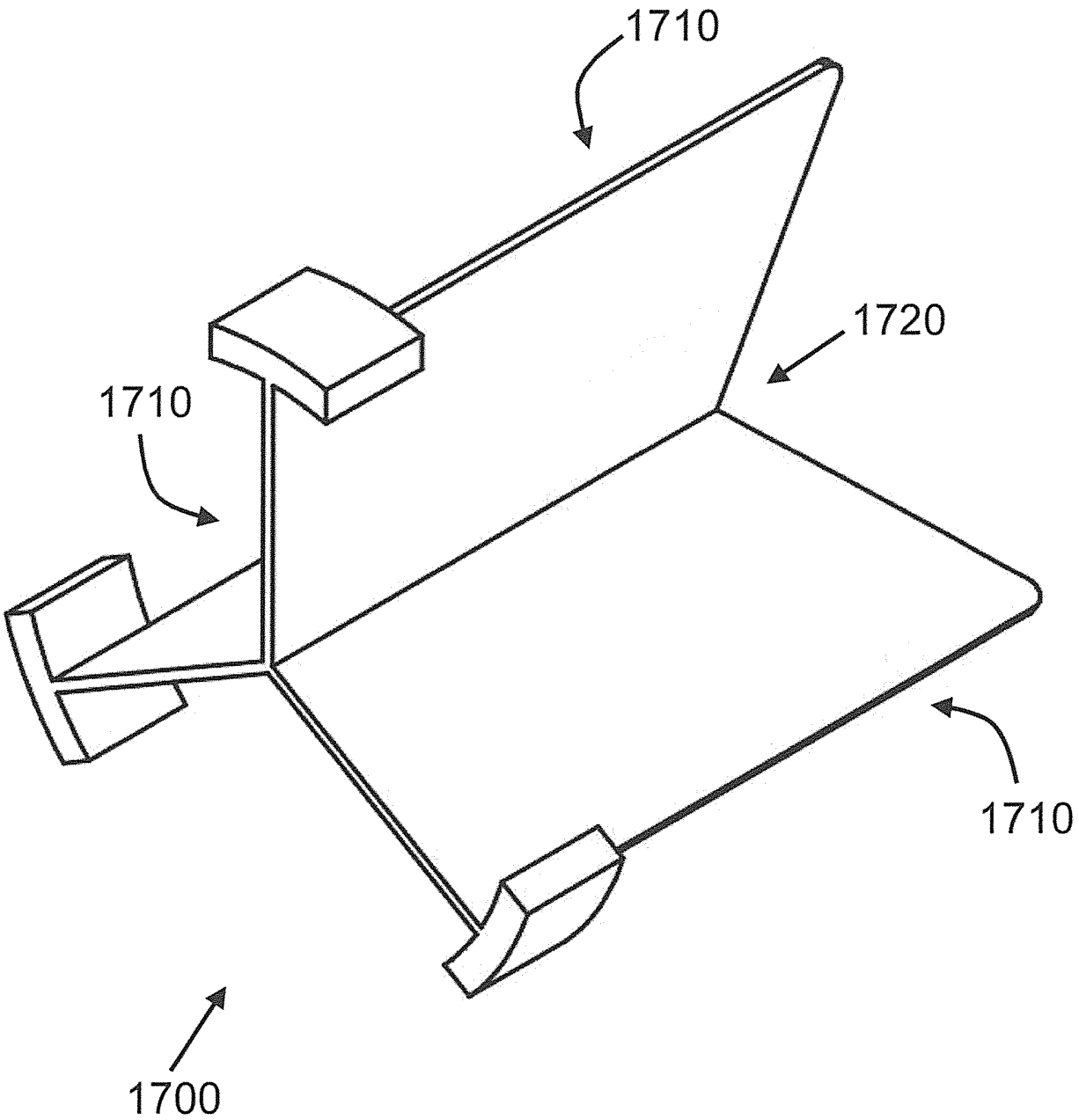

FIG. 17

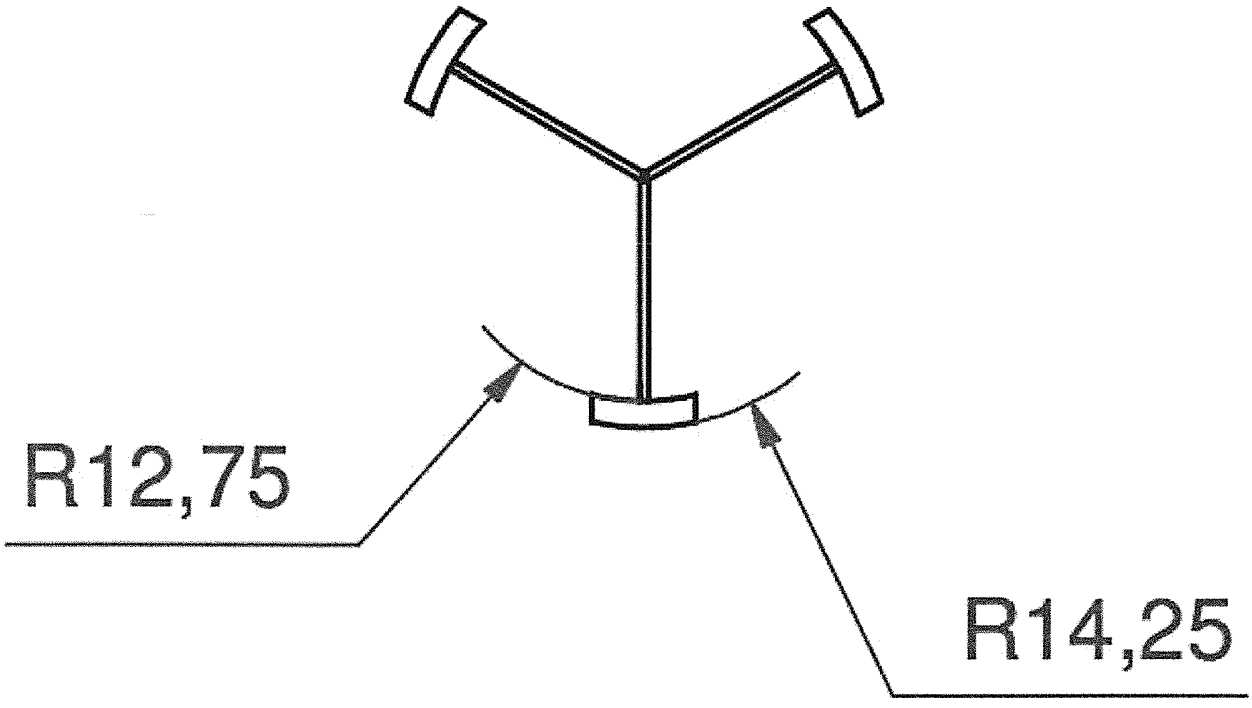

FIG. 18

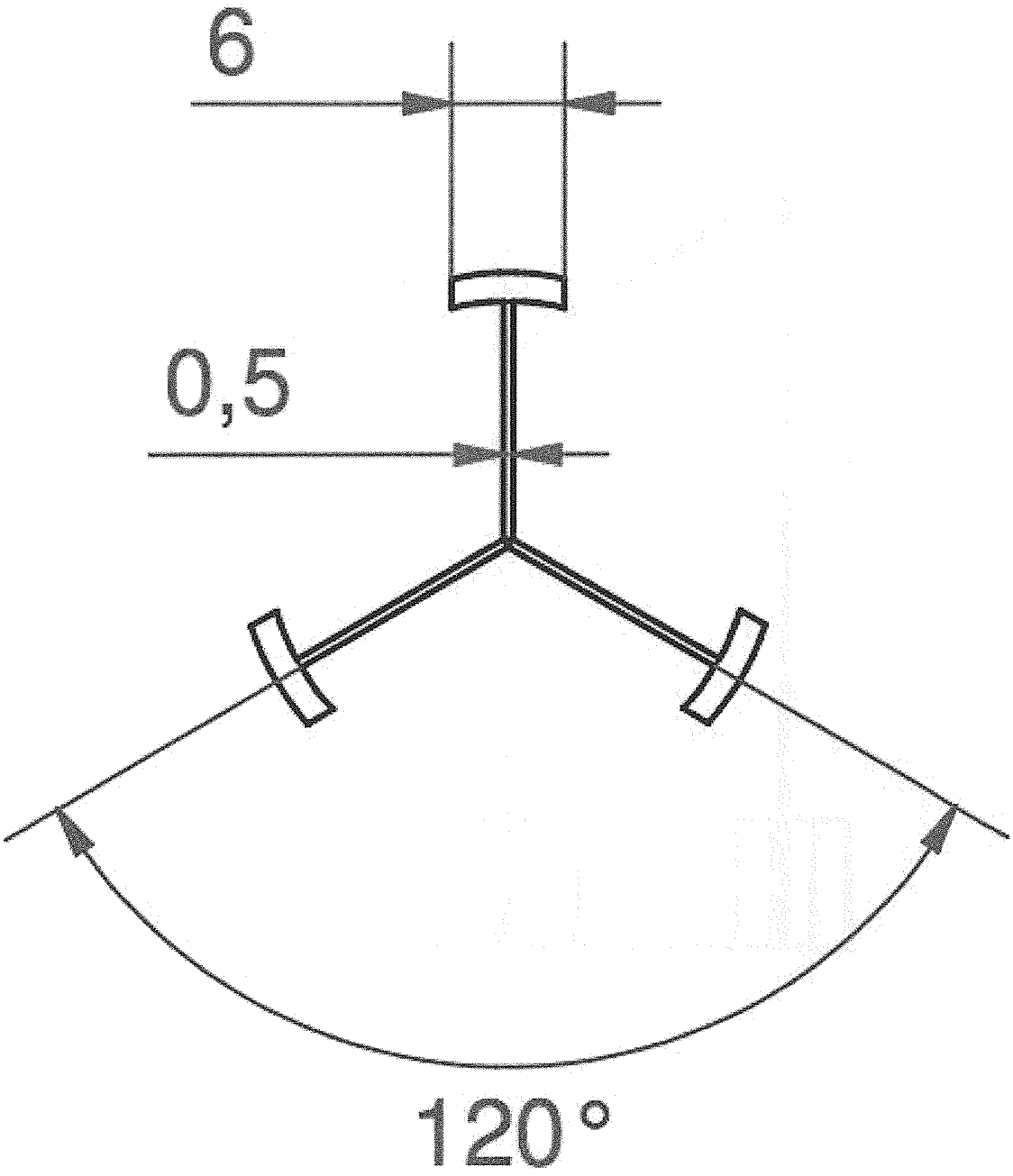

FIG. 19

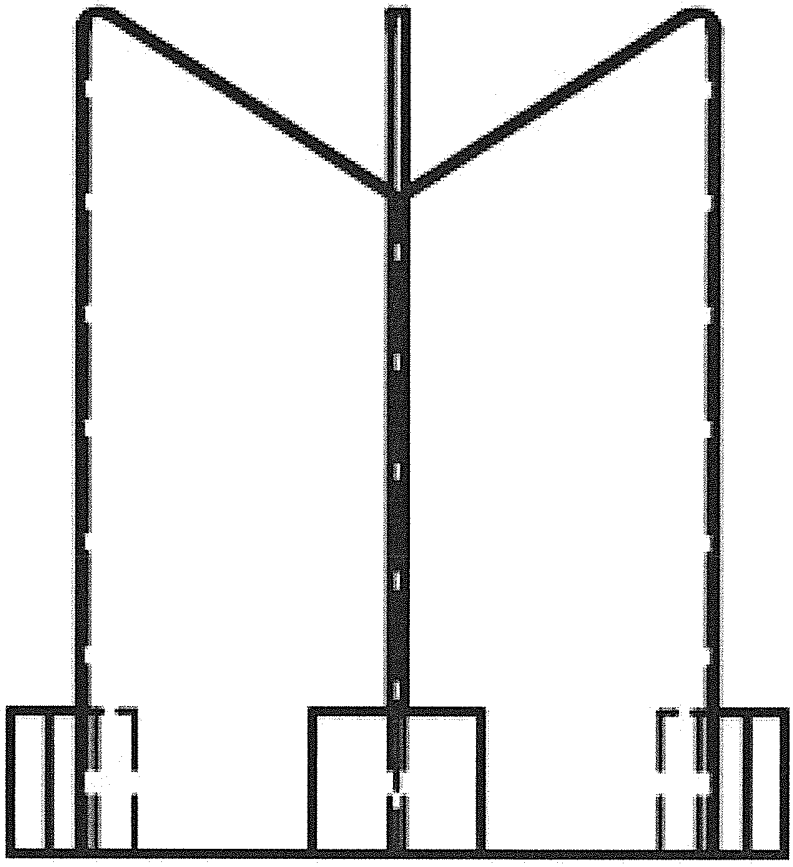

FIG. 20

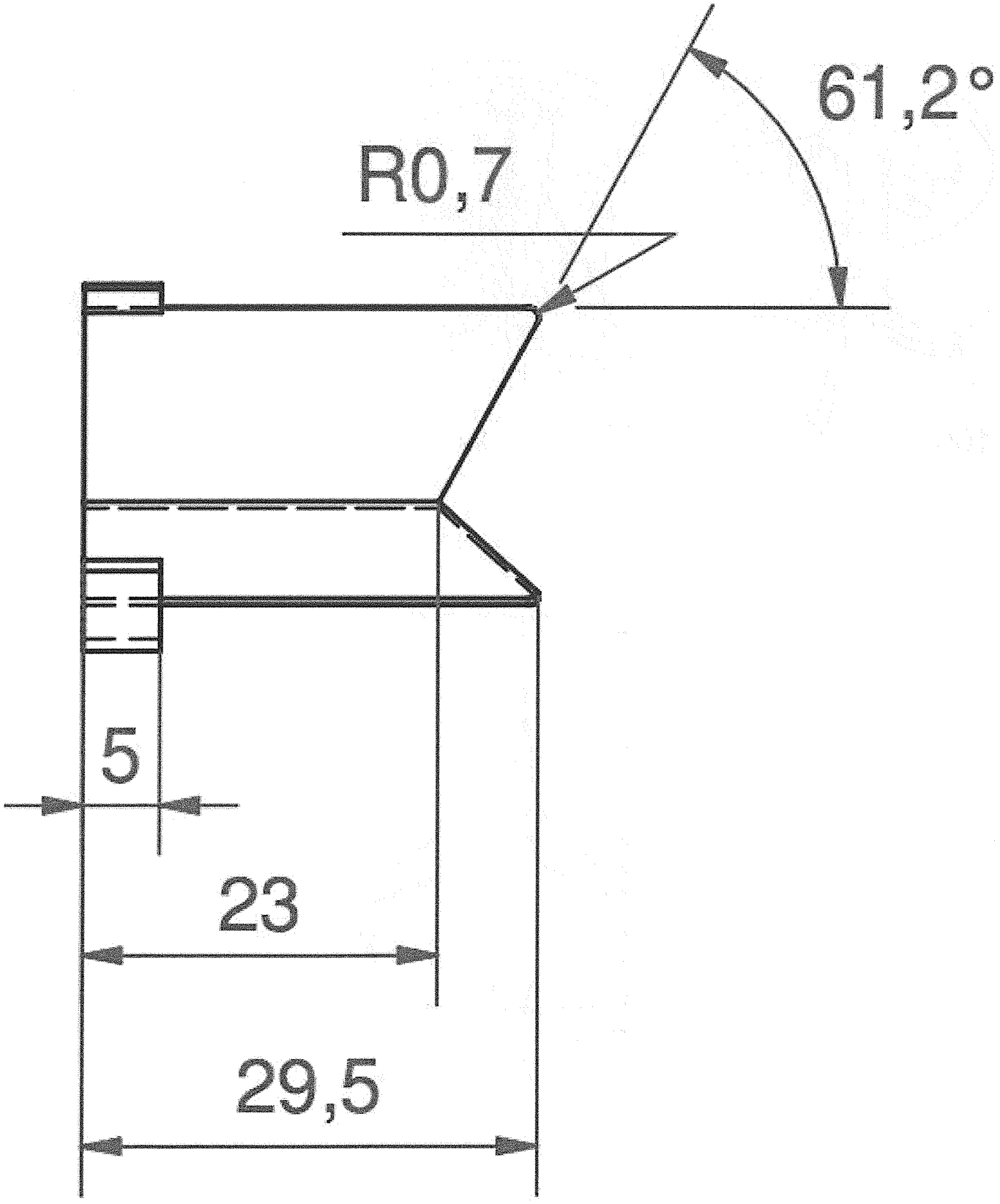

FIG. 21

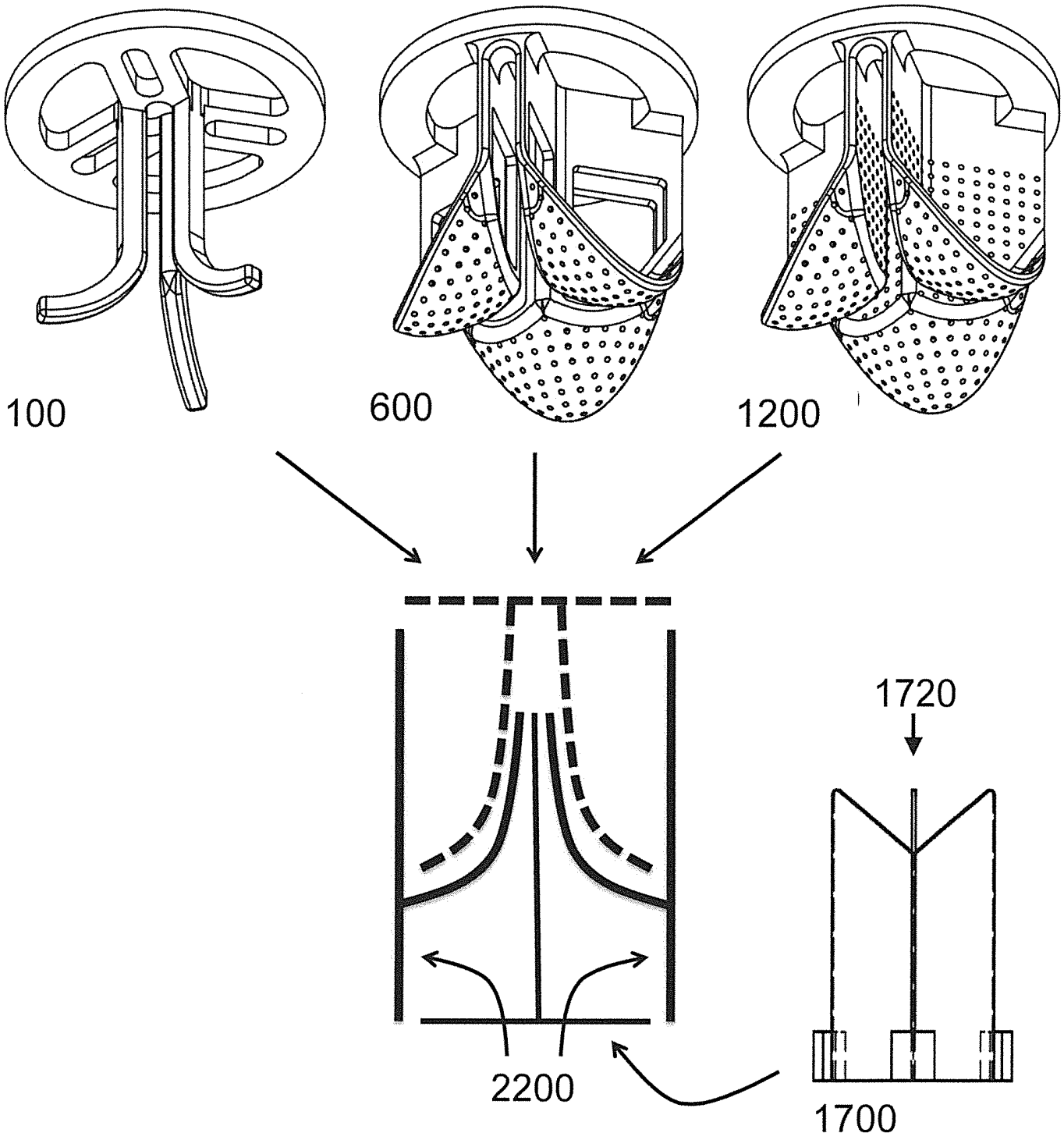

FIG. 22

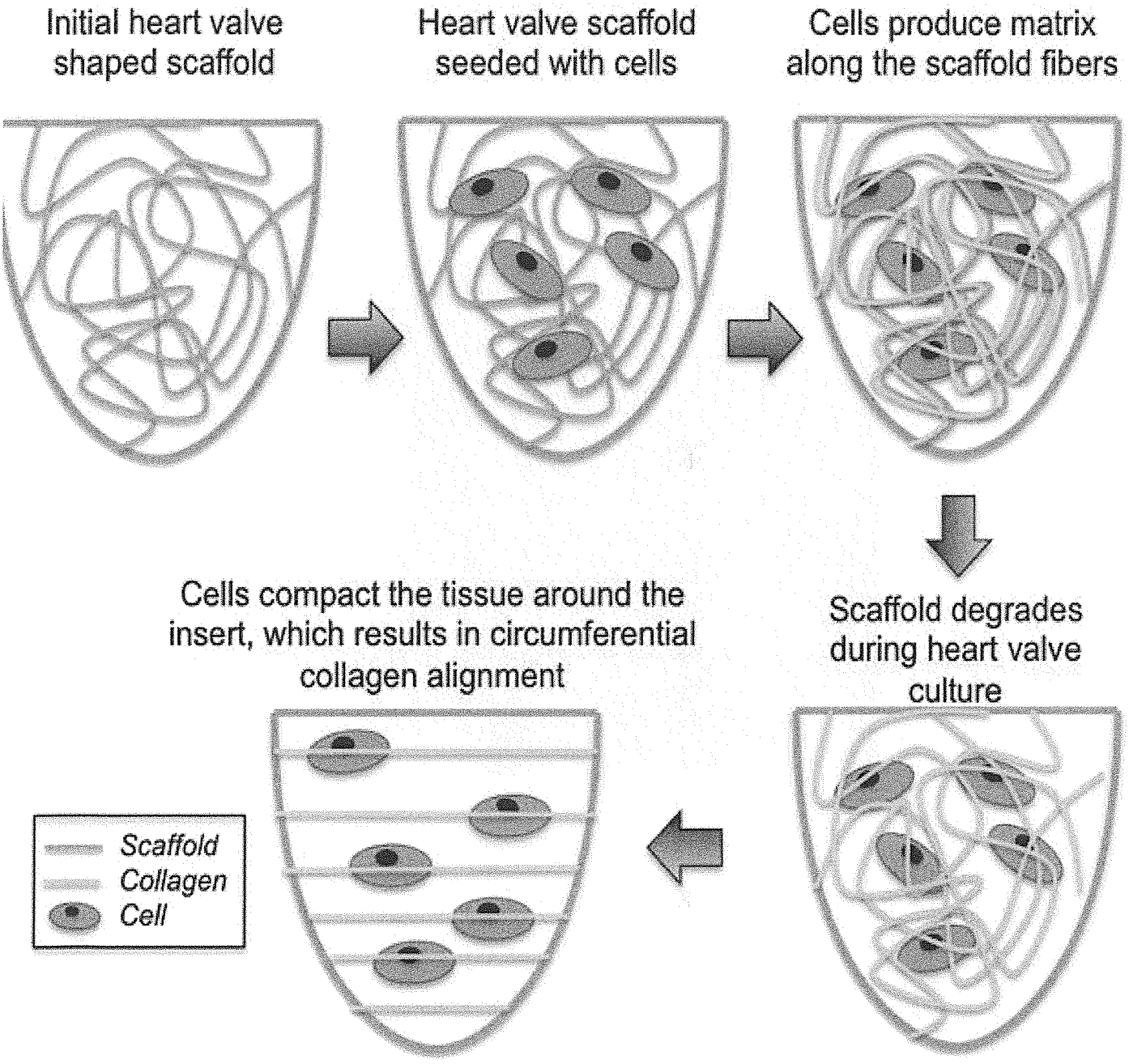
Initial heart valve shaped scaffold
Heart valve scaffold seeded with cells
Cells produce matrix along the scaffold fibers
Scaffold degrades during heart valve culture
Cells compact the tissue around the insert, which results in circumferential collagen alignment
Scaffold
Collagen
Cell

FIG. 23

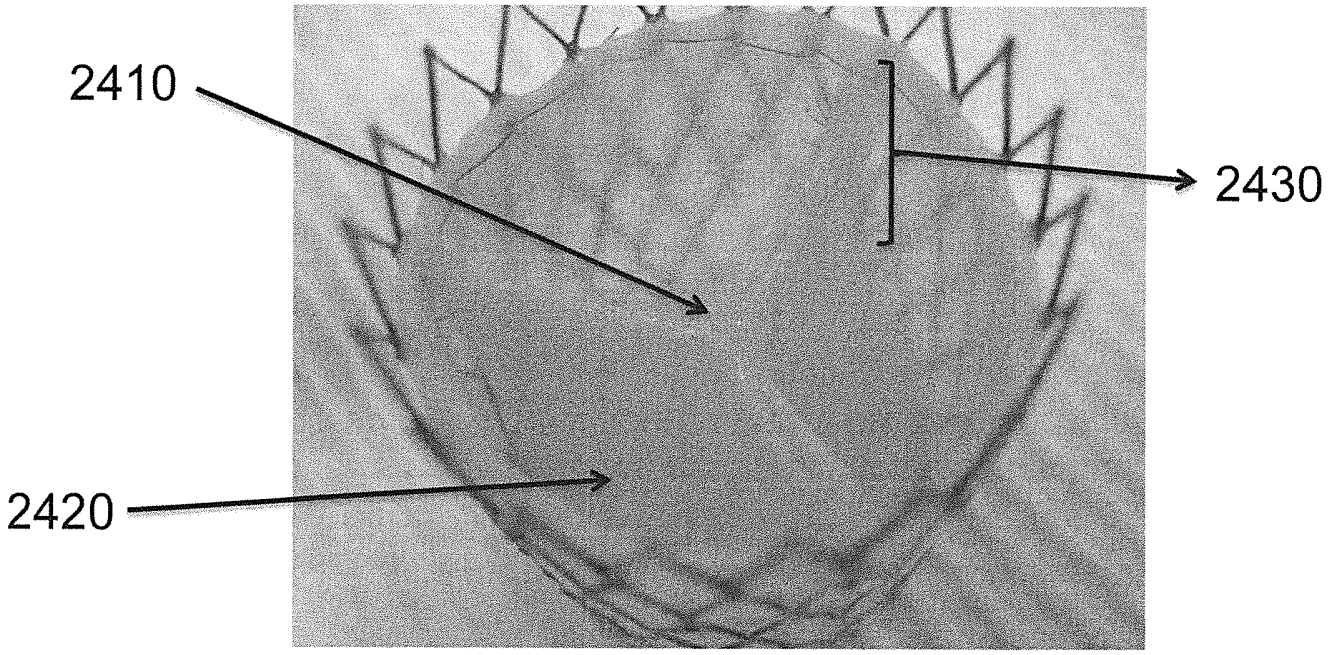

FIG. 24

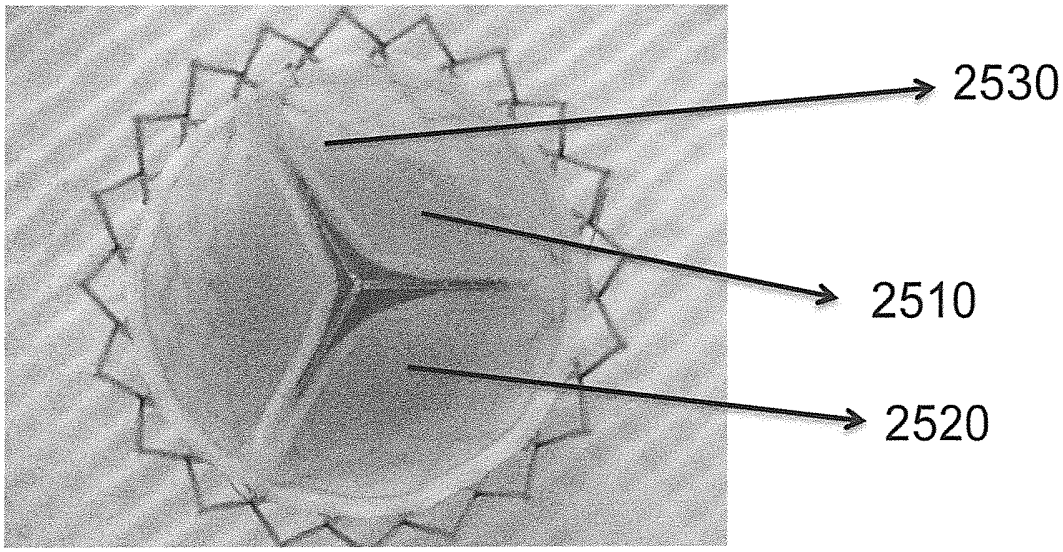

FIG. 25

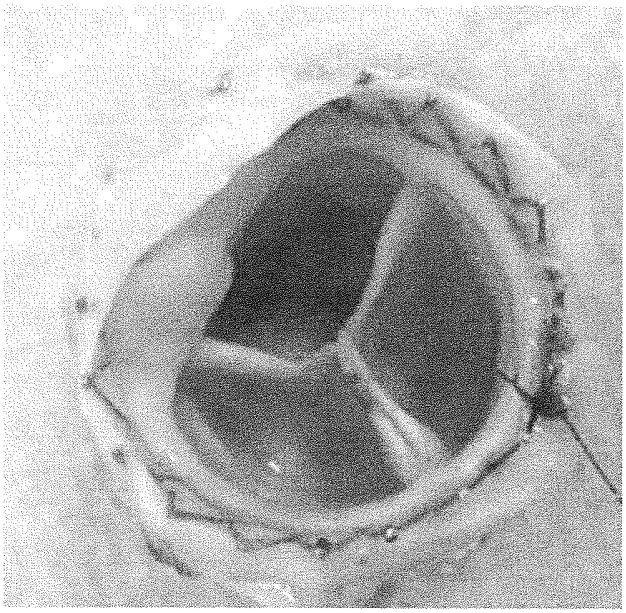

FIG. 26

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/070352

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61F2/24
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

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 243 375 A (SPARKS CHARLES HOWARD [US]) 18 August 1971 (1971-08-18) column 4, lines 6-53; figures 6-10	1-12
A	US 2012/244617 A1 (ALAVI SEYEDHAMED [US] ET AL) 27 September 2012 (2012-09-27) paragraphs [0047] - [0054]; figures 1a,1b,9a-9d	1-12
A	"ANNOUNCEMENT", CHEMISTRY & INDUSTRY, SOCIETY OF CHEMICAL INDUSTRY. LONDON, GB, no. 9, 1 May 1995 (1995-05-01), page 330, XP000505203, ISSN: 0009-3068 the whole document	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 November 2014	14/11/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Espuch, Antonio

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/070352

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 1243375 A	18-08-1971	DE 1766712 A1	23-03-1972
		FR 1575107 A	18-07-1969
		GB 1243375 A	18-08-1971
		SE 356898 B	12-06-1973
		US 3514791 A	02-06-1970
US 2012244617 A1	27-09-2012	EP 2688562 A2	29-01-2014
		KR 20140020288 A	18-02-2014
		US 2012244617 A1	27-09-2012
		US 2012245706 A1	27-09-2012
		WO 2013025239 A2	21-02-2013