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(54) **MAGNETIC SEPARATION WITH ROTATING MAGNETIC FIELD/ROTATING COLUMN**

(57) The invention is directed to a cell separation device comprising a magnet generating a magnetic field and a separation column **characterized in that** the mag-

netic field and the separation column are capable of rotating relative to each other.

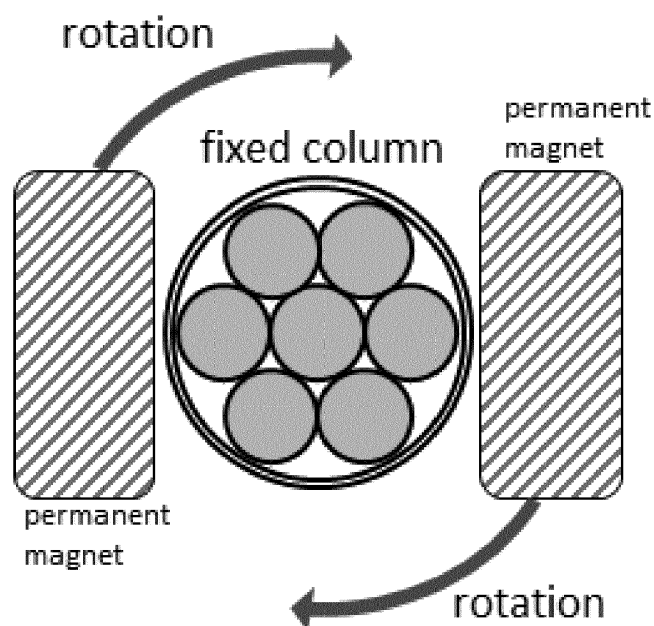


Fig. 1

Description

BACKGROUND

[0001] The invention is directed to a device and a process for magnetic cell separation comprising a magnet producing a magnetic field and a separation column wherein the magnetic fields and separation column can be rotated relative each other.

[0002] Magnetic cell separation is a long-known technology, especially under the trade name MACS of Miltenyi Biotec B.V. & Co. KG. In this technology, cells are, magnetically labelled and separated from non-magnetic cells by a high-gradient magnetic separator (columns), for example as provided by Miltenyi Biotec B.V. & Co. KG. Such process and device are disclosed for example in US6602422B1.

[0003] In order to obtain the separated cells afterwards from the column, either the column can be removed from the magnetic field as conventional MACS technology states or using "REAl ease" technology wherein the magnetic label is removed after separation from the individual cells.

[0004] In both cases, part of the cell population is still retained in the column due to cell-cell or cell-column adhesion, leading to lower yield. By using a plunger, as proposed by EP3400983B1, inducing extremely high flowrate, the remaining cell population can be completely eluted, which has however various detrimental effects. Furthermore, no further selection of cells can be made after applying plunging due to the unspecific elution.

Object of the invention

[0005] Accordingly, object of the invention was to enhance the elution efficiency of the known magnetic cell sorting devices.

[0006] Surprisingly, this was accomplished by subjecting the column with the retained target cells to a rotating magnetic field, thereby mechanically releasing adhered target cells from the column.

[0007] First object of the invention is a cell separation device comprising a magnet generating a magnetic field and a separation column **characterized in that** the magnetic field and the separation column are capable of rotating relative to each other.

[0008] The relative rotation of the magnetic field against the separation column may be accomplished by either a static separation column and a rotating magnetic field or a static magnetic field and a rotating separation column.

[0009] The term "subjecting the target cells / the separation column to a rotating magnetic field" refers to the magnetic forces interacting with the target cells / the separation column. The term refers to both embodiments, i. e. fixed (static) separation column and a rotating magnetic field or a static magnetic field and a rotating separation column.

[0010] Without being bound to this theory, the rotation results in a new orientation of the magnetic field which causes changes in spots of magnetic attraction, further leading to movements of magnetically labelled cells or magnetic micro-beads. The movements of the cells can release cells from adhering to column wall or to each other. Further movements of magnetic particles can cause increased flowrate which also facilitates the detachment of the cells during elution.

[0011] Another object of the invention is a process for magnetic separation of target cells from a sample comprising target and non-target cells wherein

- a) the sample is provided with magnetic beads which bind selective to the target cells;
- b) subjecting the sample in a separation column to a magnetic field thereby removing the non-target cells and retaining the target cells in the separation column;
- c) removing the separation column from the magnetic field and obtaining the target cells from the separation column by flushing the target cells from the separation column with a liquid

characterized in that

the retained target cells in the separation column are subjected to a rotating magnetic field obtained from the separation column by flushing the target cells from the separation column with a liquid in absence of a magnetic field .

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The drawings shall explain the invention and its embodiments without limiting the scope of the claims.

Fig. 1 shows an embodiment of the invention wherein the magnetic field rotates relative to a fixed column. Fig. 2 shows an embodiment of the invention wherein a magnet (1) generates a static (fixed) magnetic field wherein separation column (2) is rotated in the magnetic field by a mechanical power transmission mechanism (3) powered by an appropriate motor (4). Fig. 3 and 4 show variants of the invention providing a magnet capable of rotational movement relative to the separation column located between the poles (1) (not shown). The yoke is located on mechanical structure (4) providing mechanical power transmission via axial bearing (4) from motor (5).

Fig. 5 shows another variant of the invention with a rotating magnetic field provided by pairs of electromagnets (1). The magnetic field is generated by coils (2). By activating pairs of coils located at opposing sides of each other, different directions of the magnetic field can be achieved. By rotating the activation of coil pairs, a rotating magnetic field is generated. (3) indicates the separation column with iron shots (4).

DETAILED DESCRIPTION

[0013] The rotation of the magnetic field relative to the column can be achieved by rotation of the separation column in a static magnetic field or by rotation of the magnetic field relative to a static column.

[0014] In the invention, the magnetic field and the separation column may rotate relative to each in a full circle i.e. by a rotational movement in one direction (clockwise or counter-clockwise). The term "full circle" refers to movements of more than 360 degrees.

[0015] In another variant of the invention, the magnetic field and the separation column are capable of rotating relative to each other in alternating directions, for example in alternating directions for 5 to 360 degrees. The type of rotation can be continuous in clockwise or counter-clockwise direction or in alternating clockwise or counter-clockwise direction with a degree of rotation of 5 to 360 degrees, for example of 60°, 90°, 120°, 180° or 360°.

[0016] In any case, the speed of rotation can be 10 rpm to 60 rpm, preferable between 20-40 rpm. The speed of changing the rotational movement in alternating directions may be in the same magnitude like 10 to 60 rpm, preferable between 20-40 rpm.

[0017] As already pointed out, in the invention the separation column may be capable of rotating in a static magnetic field or in alternative, the magnetic field is capable of rotating relative to a static separation column.

[0018] In the first embodiment of the invention, the separation column is provided with a mechanical power transmission mechanism, for example a belt drive, a friction wheel drive or a gear wheel. An example of a mechanism of the first embodiment is shown in Fig. 2. The rotatable column can have an open end for direct pipetting (Fig. 2).

[0019] The rotatable column can be provided with a closed tubing system, wherein a rotatable adaptor or a flexible tubing section allows separate rotation of the column relative to the rest part of the tubing.

[0020] In the second embodiment of the invention, the magnetic field is capable of rotation relative to a static separation column. The static column can be provided with a (standard) closed tubing system.

[0021] To this end, the magnets and/or the yoke of the magnets may be located on a rotating platform which is driven by an electric motor via gear wheels. Fig 3 and 4 show examples of this embodiment, with open or single-sided closed yokes (2). The magnets (1) can be permanent magnets or electromagnets, providing the north and south pole of the magnetic field.

[0022] The rotational movement can be achieved by appropriate mechanical power transmission mechanism like a gear, a belt drive or a friction wheel drive or other mechanical power transmission mechanisms

[0023] In a variant of this embodiment, the magnetic field is generated by an array of electromagnets wherein the electromagnets are activated and deactivated in an alternating sequence. Here, pairs of magnets opposing

each other are switched on and off in a coordinated process generating a rotating magnetic field. A suitable number of magnets might be a pair of magnets every 30° or 60°

EXAMPLES

Example 1 - Isolation of B Cells - Elution without plunger and rotation of the column during the elution in a static magnetic field

[0024] Peripheral blood mononuclear cells (PBMC) were prepared from buffy coat preparations from human whole blood. The current state-of-the-art reagent for isolation of B Cells "REAl ease® CD19 MicroBead Kit, human" (MiltenyiBiotec) was used. 1×10^7 PBMC was labelled with the reagents accordingly to the protocol. The cells were applied to a prepared high gradient magnetic column (HGMC) and a wash was proceeding with 3 times 0,5mL buffer. To elute the cell of interest, two times 7mL of the REAl ease® Bead Release buffer was applied to the column. While the buffer runs through the column, the column was rotated with 30rpm. The flowthrough was collected as target fraction. Residual cells were eluted by using the plunger and 1mL PBS buffer and collected as plunger fraction. The sum of the amount of CD19 positive cells within the target fraction and CD19 positive cells within plunger fraction represents the total amount of isolated CD19 positive cells.

[0025] The efficiency of the elution without plunger was calculated by the amount of CD19 positive cells divided by the total amount of isolated CD19 positive cells.

[0026] As a control the elution of cell of interest was performed without rotation of the column. The mean efficiency of the elution with rotation was calculated with 93,1%. The mean efficiency of the elution without rotation was calculated with 82,1%.

Example 2 - Isolation of B Cells - Elution without plunger and short rotation of the column during the elution in a static magnetic field

[0027] PBMC were prepared from buffy coat preparations from human whole blood. The current state-of-the-art reagent for isolation of B Cells "REAl ease® CD19 MicroBead Kit, human" (MiltenyiBiotec) was used. 1×10^7 PBMC was labelled with the reagents accordingly to the protocol. The cells were applied to a prepared HGMC and a wash was proceeding with 3 times 0,5mL buffer. To elute the cell of interest, 7mL of the REAl ease® Bead Release buffer was applied to the column. After the REAl ease® Bead Release buffer runs through the column, the column was rotated 30 seconds with 30rpm. 7mL of the REAl ease® Bead Release buffer was applied to the column. Both flowthroughs were collected as target fraction. Residual cells were eluted by using the plunger and 1mL PBS buffer and collected as plunger fraction. The sum of the amount of CD19 positive cells within the target

fraction and CD19 positive cells within plunger fraction represents the total amount of isolated CD19 positive cells. The efficiency of the elution without plunger was calculated by the amount of CD19 positive cells divided by the total amount of isolated CD19 positive cells.

[0028] As a control the elution of cell of interest was performed without rotation of the column. The mean efficiency of the elution with rotation was calculated with 88,5%. The mean efficiency of the elution without rotation was calculated with 81,2%.

Example 3 - Isolation of CD4 positive T Cells - Elution without plunger and rotation of the column in a static magnetic field

[0029] PBMC were prepared from buffy coat preparations from human whole blood. The current state-of-the-art reagent for isolation of B Cells "REAl ease[®] CD4 MicroBead Kit, human" (MiltenyiBiotec) was used. 1×10^7 PBMC was labelled with the reagents accordingly to the protocol. The cells were applied to a prepared HGMC and a wash was proceeded with 3 times 0,5mL buffer. To elute the cell of interest, two times 7mL of the REAl ease[®] Bead Release buffer was applied to the column. While the buffer runs through the column, the column was rotated with 30rpm. The flowthrough was collected as target fraction. Residual cells were eluted by using the plunger and 1mL PBS buffer and collected as plunger fraction. The sum of the amount of CD4 positive cells within the target fraction and CD4 positive cells within plunger fraction represents the total amount of isolated CD4 positive cells. The efficiency of the elution without plunger was calculated by the amount of CD4 positive cells divided by the total amount of isolated CD4 positive cells.

[0030] As a control the elution of cell of interest was performed without rotation of the column. The mean efficiency of the elution with rotation was calculated with 98,5%. The mean efficiency of the elution without rotation was calculated with 95,3%.

Claims

1. Cell separation device comprising a magnet generating a magnetic field and a separation column **characterized in that** the magnetic field and the separation column are capable of rotating relative to each other.
2. Cell separation device according to claim 1 **characterized in that** the magnetic field and the separation column are capable of rotating relative to each in a full circle.
3. Cell separation device according to claim 1 **characterized in that** the magnetic field and the separation column are capable of rotating relative to each other

in alternating directions.

4. Cell separation device according to claim 3 **characterized in that** the magnetic field and the separation column are capable of rotating relative to each other in alternating directions for 5 to 360 degrees.
5. Cell separation device according to any of the claims 1 to 4 **characterized in that** the separation column is capable of rotating in a static magnetic field.
6. Cell separation device according to any of the claims 1 to 5 **characterized in that** the magnetic field is capable of rotating relative to a static separation column.
7. Cell separation device according to claim 6 **characterized in that** the magnetic field is generated by an array of electromagnets wherein the electromagnets are activated and deactivated in an alternating sequence.
8. Process for magnetic separation of target cells from a sample comprising target and non-target cells wherein

d) the sample is provided with magnetic beads which bind selective to the target cells;

e) subjecting the sample in a separation column to a magnetic field thereby removing the non-target cells and retaining the target cells in the separation column;

f) removing the separation column from the magnetic field and obtaining the target cells from the separation column by flushing the target cells from the separation column with a liquid

characterized in that

the retained target cells in the separation column are subjected to a rotating magnetic field obtained from the separation column by flushing the target cells from the separation column with a liquid in absence of a magnetic field.

9. Process according to claim 9 **characterized in that** the magnetic beads from the retained target cells are removed thereby obtaining un-labelled target cells; and removing the unlabelled target cells from the separation column by subjecting the target cells to a rotating magnetic field; and flushing the target cells from the separation column with a liquid in absence of a magnetic field
10. Process according to claim 8 and 9 **characterized in that** the target cells are subjected to a rotating magnetic field and flushed from the separation column with a liquid in absence of a magnetic field in 2 to 10 cycles.

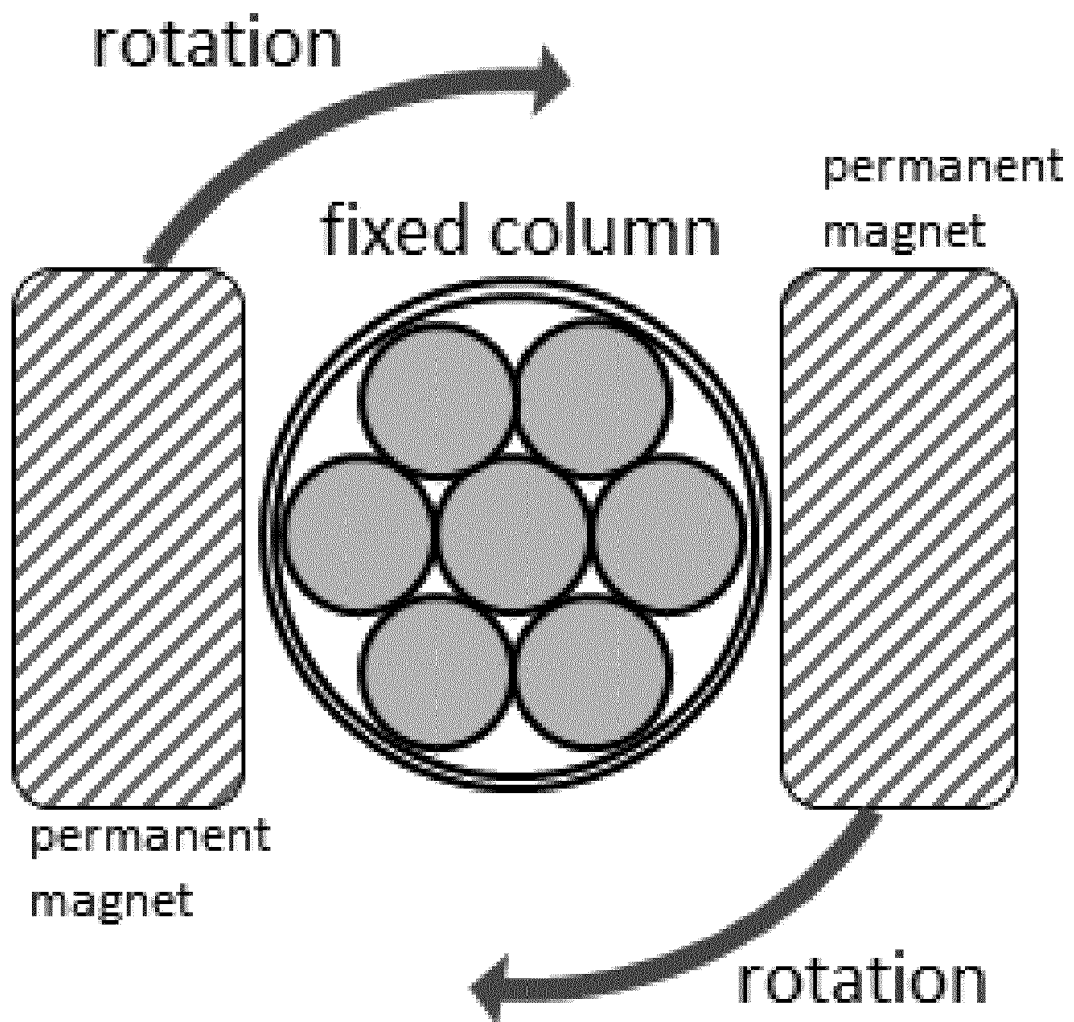


Fig. 1

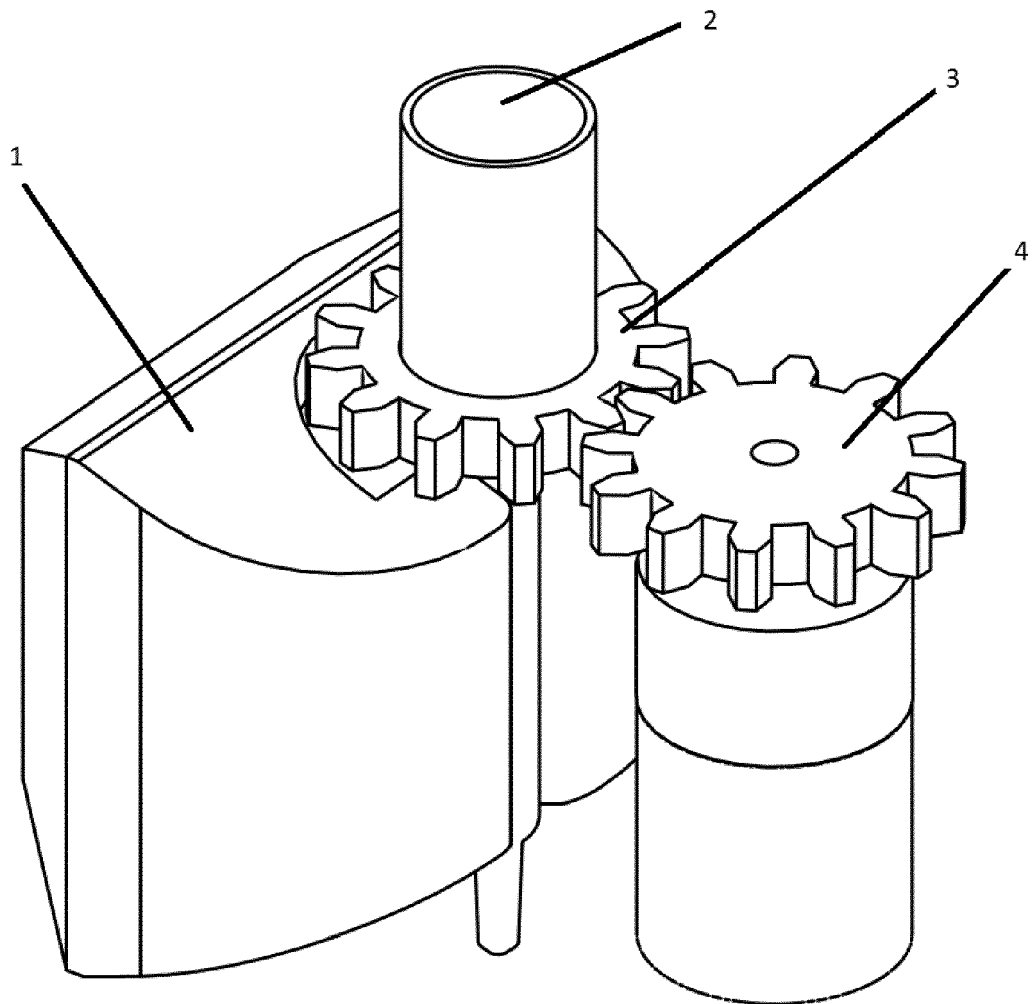


Fig. 2

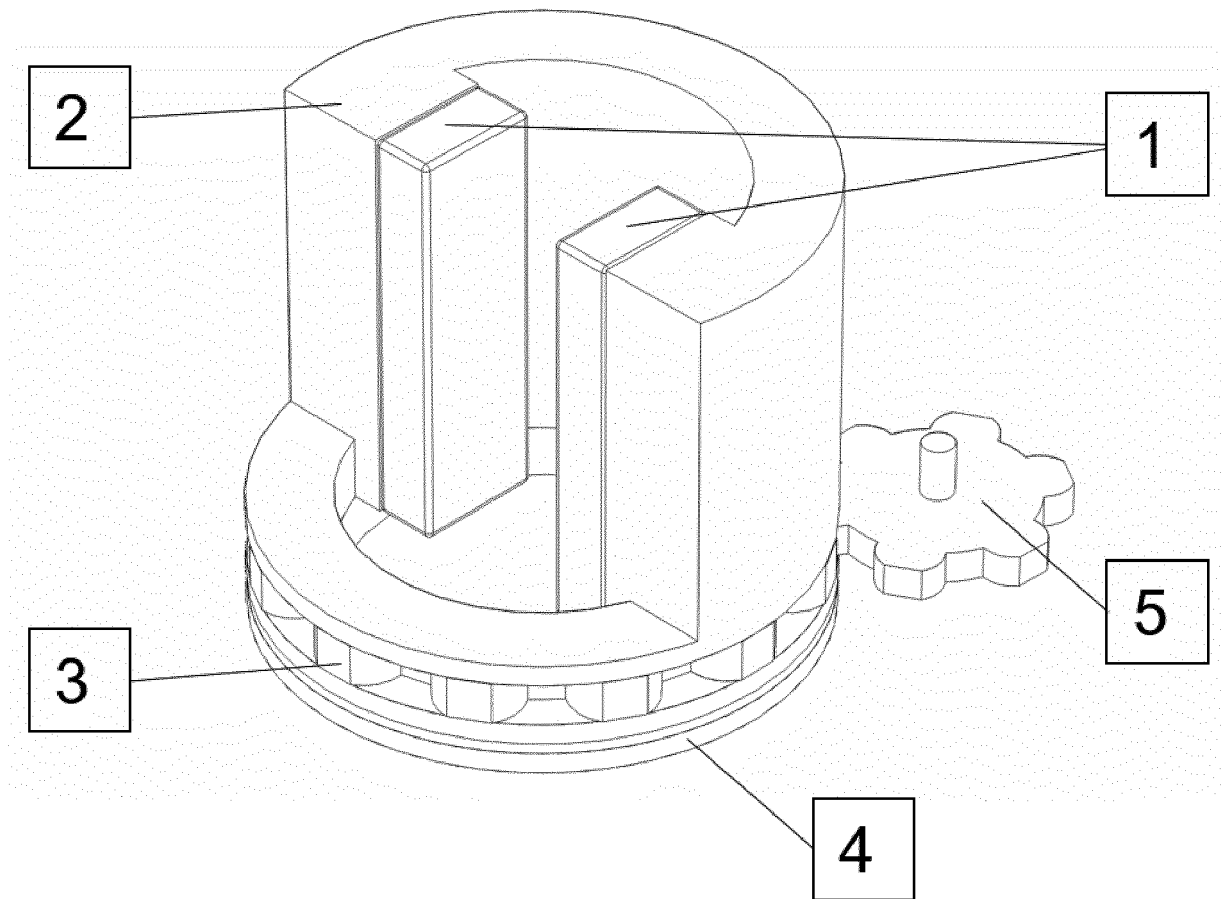


Fig. 3

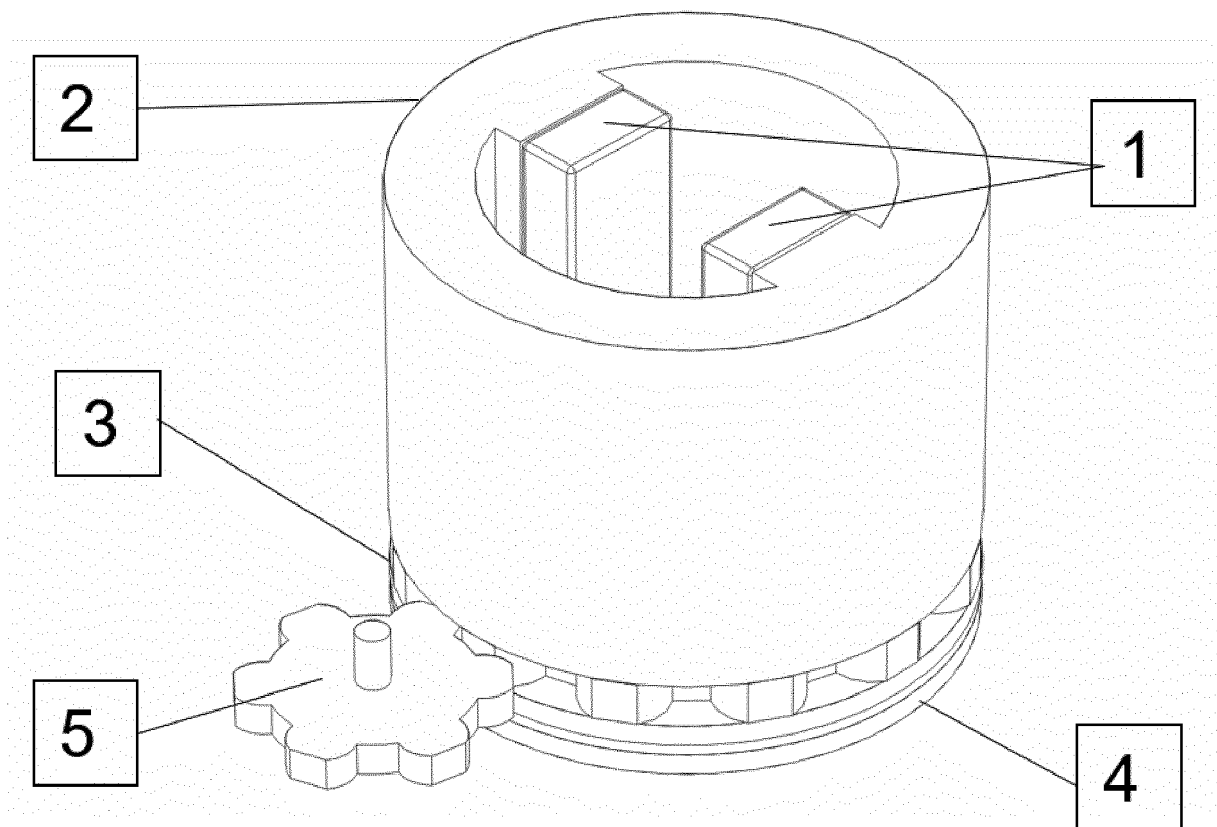


Fig. 4

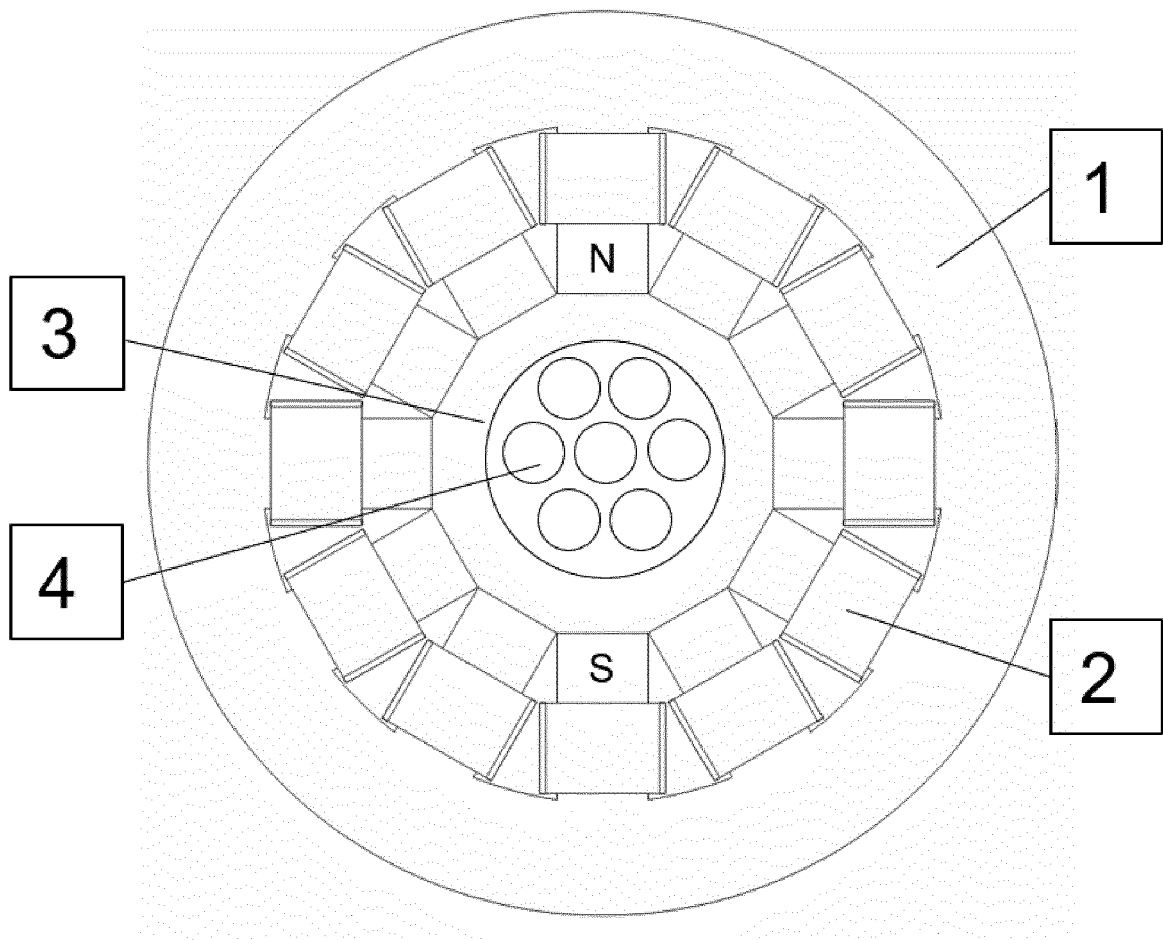


Fig. 5



EUROPEAN SEARCH REPORT

Application Number

EP 21 18 4700

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Place of search The Hague		Date of completion of the search 14 December 2021	Examiner Menck, Anja
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

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