

## DESCRIPTION

### FLUIDIC CHIP AND CELL ENCAPSULATION SYSTEM

5 [0001] The present invention relates to the technical field of fluidic chips and cell encapsulation systems comprising such a fluidic chip.

[0002] A fluidic chip is a set of channels etched or molded in a material such as glass, silicon or a polymer such as PDMS for PolyDiMethylSiloxane.

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[0003] In the above domain, fluidic chips are known comprising inlets for receiving various substances and a single outlet through which the substances are thus combined. Fluidic chips comprise channels with sections whose dimensions are of the order of a millimeter to a micrometer which connect the inlets to the outlet.

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[0004] Such fluidic chips can be manufactured by means of various techniques. The most common manufacturing technique comprises steps of molding the "PDMS" material or by embossing a thermoplastic material or by injection-molded thermoplastic or even also by means of a chemical lithography  
20 technique or a resin printing technique.

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[0005] Most fluidic chips are used with liquid-liquid interfaces, between two liquids most often immiscible and have a two-dimensional architecture sufficient for this use. These interfaces can be used to make chemical reactions for example (microreactors) but are often used to form beads or capsules. When they use liquid-liquid interfaces, fluidic chips are only rarely used to form jets and most of the time use dripping regimes. In order to form a stable jet in the air at the outlet of the chip, a 3D architecture is necessary. As for all fluidic chips, and even more so for their internal structures, great  
30 precision is necessary to avoid instabilities.

[0006] For the production of 3D or three-dimensional fluidic chips, the main technique is based on the printing of 3D photopolymerizable resins. Two types of machines can then be used: widely marketed stereolithography printers such as "Formlab" or "Envisiontech" printers and femtosecond laser polymerization.

5 Whereas the first technique makes it possible to produce objects several centimeters in length, the resolution only goes down to about fifty microns and does not make it possible to produce robust and reproducible structures less than a few hundred micrometers long. The second technique benefits from a very high spatial resolution of less than one micrometer but is not industrially usable for the  
10 production of parts comprising dimensions in the order of one centimeter. In addition, these techniques have the disadvantages of polymerizable resins which can make them unsuitable for their use in certain fields such as bio-production, due to their chemical composition or even lack of certification.

15 [0007] A technique for the production of 3D fluidic chips is based on 3D-subtractive printing of glass (Selective Laser Etching) of molten silica, quartz, sapphire or borosilicate type. A laser thus irradiates the parts to be removed, to form channels in a glass plate after chemical visualization. The technique is intrinsically limited in terms of height due to the limited working distance of  
20 the optical system, all the more so since the precision to be achieved is large. The irradiated parts are then dissolved in an acidic or basic chemical bath. This method can last several days, hence the benefit of also limiting the length of the channels to avoid remaining in the bath too long. In addition to the impact on the productivity and speed of the process, excessively long  
25 bath times can create excessive geometric and dimensional alterations of the structures close to the inlets and outlets. In addition, according to this method, the cost of the part produced is proportional to the dimension along the central axis Z. Finally, the presence of long channels in the chips causes a greater pressure drop, which represents a real disadvantage in the use  
30 according to the application and the liquids used.

[0008] It is then necessary to find a way of compacting the dimensions along the central axis Z.

5 [0009] The method described above is used for manufacturing 3D fluidic chips for the production of a "shell-shell" type structure by double emulsion, such as "Raydrop" technology. Such chips comprise a 3D-printed glass head mounted on two coaxial capillaries thus enabling a drop-wise encapsulation in 3D. Limited by printing along the Z axis and the cost, only the head is 3D-printed, which therefore adds a complex mounting step to maintain a certain  
10 precision of coaxial capillaries. This type of structure is always in the liquid phase comprising immiscible liquids such as oil and water. The use of two coaxial capillaries represents a real fluid resistance which can represent a real limitation in its use, depending on the flow rates and fluids used.

15 [0010] Depending on the flow rates and the nature of the substances injected into each of the inlets of the fluidic chip, instability may appear in the various fluids flowing through the channels of the fluidic chip. Unlike dripping regimes, the production of jets requires greater flow rates which makes them more sensitive to instabilities, even more so for jets in the air rather than in  
20 another non-miscible liquid such as oil.

[0011] The invention thus aims to overcome these various problems and proposes a simple-to-make fluidic chip for obtaining a coaxial jet at the outlet of the chip consisting of the various incoming substances. The invention makes it  
25 possible to compact the dimensions along the Z axis while ensuring a stable coaxial jet and without the need for a coaxial incoming injection of the fluids.

[0012] The invention therefore relates to a fluidic chip composed of a body comprising a main inlet and at least one secondary inlet not coaxial to the main  
30 inlet, each main inlet and secondary inlet being intended to receive a substance and an outlet, the main inlet is connected to the outlet by a main channel

comprising a substantially rectilinear portion defining a central axis Z for the fluidic chip, the secondary inlet is also connected to the outlet by a secondary channel.

5 [0013] The invention therefore relates to a fluidic chip for obtaining a coaxial jet in a gas at the outlet composed of a body comprising a main inlet and at least one secondary inlet not coaxial to the main inlet, each main inlet and secondary inlet being intended to receive a substance and an outlet, the main inlet is connected to the outlet by a main channel comprising a substantially rectilinear portion defining a central axis Z for the fluidic chip, the secondary  
10 inlet is also connected to the outlet by a secondary channel, the geometry of the main channel and secondary channel is three-dimensional.

[0014] According to one embodiment, the coaxial jet at the outlet of the fluidic chip is made in the air. Other gaseous environments are compatible with the invention.  
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[0015] According to the invention, the secondary channel is divided into at least two secondary ducts of the same dimension that join in a circular injection duct coaxial with the main channel in an evenly distributed manner about the central axis Z. The implementation of secondary ducts of the same hydraulic resistance  
20 makes it possible to homogeneously divide the flow of substance injected into the corresponding secondary inlet while maintaining stability of the substance within and at the outlet of the chip. All the secondary inlets comprise secondary channels that divide into secondary ducts of the same dimension, and therefore also of the same cross-section and of the same shape within the same secondary channel.

25 [0016] All the secondary ducts coming from the same secondary inlet join in a circular injection channel, coaxial to the main channel, in an evenly distributed manner relative to the main axis and at the same coordinate along the central axis Z in order to ensure homogeneous injection of the flows about  
30 the central axis.

[0017] The three-dimensional geometry of the main channel and secondary channel makes it possible to increase the stability of the coaxial jet at the outlet. Indeed, the distribution of the secondary channel(s) around the main channel at the outlet of the fluidic chip is a coaxial distribution in three dimensions.

5 Advantageously, the three-dimensional structure of the fluidic chip according to the invention ensures a stable coaxial jet in the air, in particular from viscous fluid.

[0018] Such an architecture of the three-dimensional fluidic chip means that a significant portion of the flows passing through the main channel and

10 secondary channel have components in the three dimensions of the space, i.e. said flows cannot be contained in discrete planes, in particular, they cannot be contained in discrete parallel planes.

[0019] The secondary injection ducts coming from the same secondary inlet join

15 the main channel at the same coordinate along the central axis Z, or at different coordinates, allowing the creation of a flow composed coaxially of the various substances injected through the various main and secondary inlets. The creation of such coaxial jets makes it possible to obtain an isotropic flow according to the outlet XY dimensions and thus to avoid instabilities. The secondary injection

20 channels coming from different secondary inlets do not necessarily join the main channel at the same coordinate along the central axis Z.

[0020] At the outlet of the chip, at any points of the flow that are symmetrical relative to the outlet axis, the properties of the stream are symmetrical relative

25 to the outlet axis.

[0021] For any points of the symmetrical flow at the outlet relative to the outlet axis, the minimum path between the points and their entry into the chip is substantially equal.

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[0022] According to certain embodiments of the fluidic chip, a non-rectilinear portion not aligned with the central axis connects the main inlet to the main

channel. This makes it possible to place the inlets along an axis different from the central axis Z.

[0023] According to one feature of the invention, the fluidic chip is obtained by a technique of subtractive 3D printing on glass ("Selective Laser Etching"). The glass used is of the molten silica, quartz, sapphire or borosilicate type, and preferably made of molten silica. According to one embodiment, the fluidic chip is obtained by a subtractive 3D printing technique on a material allowing it. Such a material when it is removed from the material is capable of keeping great precision for the dimension of the channels while allowing the production of a part with a millimetric volume which can be greater than 1 mm<sup>3</sup> and preferably greater than 10 mm<sup>3</sup>, 100 mm<sup>3</sup> or even 1000 mm<sup>3</sup>.

[0024] According to one embodiment of the invention, the body of the fluidic chip comprises an upper face comprising the main inlet and at least the secondary inlet and a lower face opposite the upper face and comprising the outlet. The main inlet is thus aligned with the main channel and the outlet of the chip.

[0025] According to one feature of the invention, the geometry of the main channel and secondary channel is three-dimensional. Thus, the geometry cannot be complete in less than three two-dimensional planes.

[0026] According to another feature of the invention, the geometry of the fluidic chip is compact along the outlet axis. This means that the dimension of the set of channels along the outlet axis is not more than twice as much as the dimensions of all of the channels along the planes perpendicular to the outlet axis. It is thus possible to produce a fluidic chip with a dimension that does not exceed 5 cm along the Z axis and, according to other embodiments, the dimension along the Z axis of the fluidic chip may be 1 cm or less.

[0027] According to one embodiment of the invention, the outlet axis coincides with the central axis Z.

[0028] According to one feature of the invention, the secondary channel comprises a first separation at an ordinate B of the central axis Z and a second separation at an ordinate D of the central axis Z, thus defining four secondary  
5 ducts of the same length. The implementation of a secondary channel comprising four secondary ducts of the same dimension and the same hydraulic resistance allows a maximum optimization of the occupancy of the height of the fluidic chip while ensuring a coaxial jet that is stable at the outlet.

10 [0029] According to another feature of the invention, the secondary channel comprises at least one rectilinear portion opening at the center of a portion in the shape of an arc of a circle extending in a plane perpendicular to the central axis Z. Using circular arc separations to form the secondary ducts makes it possible to obtain a tree architecture and therefore equivalent paths  
15 for the flow passing through the secondary channel. In addition, such symmetry relative to the central axis Z, a coaxial axis for the fluidic chip, makes it possible to increase the stability of the flows.

[0030] According to one embodiment of the invention, the fluidic chip  
20 comprises a first and a second secondary inlet connected respectively to a first and a second secondary channel, the first secondary channel comprising a portion in the shape of an arc of a circle of diameter D1 relative to the central axis Z extending in a plane perpendicular to the central axis Z, and the second secondary channel comprising a portion in the form of an arc of a circle of  
25 diameter D2 relative to the central axis Z extending in a plane perpendicular to the central axis Z, with the diameter D1 being less than the diameter D2.

[0031] Thus, the respective secondary ducts of each of the secondary inlets extend along circular arcs of different diameters. This architecture in  
30 particular has the advantage of not crossing the secondary ducts while retaining the equivalent path principle for each secondary channel. The respective secondary ducts therefore have different dimensions depending

on the corresponding secondary channel but for the same secondary channel, each of the secondary ducts corresponding to the same dimension.

5 [0032] According to a feature of the invention, the first and second secondary channels each comprise two circular arc-shaped portions at two different ordinates from the central axis Z thus each defining four secondary ducts of the same size. The separations are thus carried out at the same ordinate, which facilitates the manufacture of the fluidic chip.

10 [0033] According to one embodiment of the invention, the main channel comprises four main ducts of the same size. The implementation of four main ducts in particular makes it possible to stabilize the flow passing through the main channel.

15 [0034] According to another feature of the invention, each main channel and secondary channel comprises a circle-shaped portion opening onto the outlet, each circle-shaped portion being concentric. This architecture has the advantage of creating coaxial jets at the outlet of the fluidic chip, which promotes certain methods of using the fluidic chip requiring concentric fluids.

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[0035] The invention also relates to a system for encapsulating cells, the system comprising at least two containers, one of the containers comprising a solution of cells and the other of the containers comprising a solution capable of gelling, an encapsulation device comprising a fluidic chip according to the invention, the  
25 main inlet and secondary inlet each being connected to one of the containers via a dispenser and able to form a concentric jet from the solutions provided by the dispensers, the encapsulation device being arranged so that the jet is divided, at the outlet of the encapsulation device, into drops, of which the outer layer is the solution capable of gelling and the core is the solution of cells, and  
30 a gelling bath arranged downstream of the encapsulation device to collect the drops formed by the encapsulation device and arranged to cause a gelling of the outer layer of each drop during its immersion in the bath.

[0036] The use of such a fluidic chip promotes the high-throughput cell encapsulation system (compared to drop-by-drop), in particular thanks to the creation of a concentric jet at the outlet of the fluidic chip according to the invention. The fluidic chip according to the invention can also be used for a drop-by-drop outlet.

[0037] The use of a fluidic chip according to the invention makes it possible to form a coaxial jet which divides into drops at the outlet of the encapsulation device and therefore promotes a three-dimensional culture system. Such drops contain in the core thereof the solution of cells which is completely surrounded by the gelling solution. These drops are then collected in a gelling bath for stiffening the outer layer thereof to form a shell.

[0038] Three-dimensional cultures are closer to natural, in vivo systems, and can be used for numerous applications, in particular in the development of treatments. For example, for a culture of stem cells, the solution capable of gelling comprises alginate and the gelling bath comprises calcium. The encapsulated cells formed in this way make it possible to cultivate cells in a liquid medium, while the shell protects the cells from mechanical stresses linked to collisions or fusions during culturing in liquid suspension.

[0039] According to one variant, the solution of cells may contain the culture medium and/or an extracellular matrix and/or an extracellular matrix substitute and/or an aqueous solution. According to another variant, the intermediate solution may contain an extracellular matrix and/or an extracellular matrix substitute. Where necessary, the encapsulation device will be arranged to form, in the collection tank, cellular microcompartments, the outer layer of which is the solution capable of gelling, an intermediate layer forming a cellular matrix or extracellular matrix substitute and the core being the solution of cells. This cellular matrix allows the cells of the solution of cells to develop and multiply. For example, the extracellular matrix substitute may comprise a mixture of proteins and

extracellular compounds required for cell culture, and more particularly pluripotent cells. Preferably, the extracellular matrix or extracellular matrix substitute may comprise structural proteins, such as laminins containing  $\alpha 1$ ,  $\alpha 4$  or  $\alpha 5$  sub-units,  $\beta 1$  or  $\beta 2$  sub-units, and  $\gamma 1$  or  $\gamma 3$  sub-units, entactin, vitronectin, laminins, collagen, as well as growth factors such as TGF-beta and/or EGF. The extracellular matrix can be an aqueous solution and/or a hydrogel, preferably a hydrogel, different from the hydrogel forming the outer layer, such as for example a hydrogel comprising or consisting of alginate, fibrin, laminin, fibronectin, entactin, hyaluronic acid and/or collagen. It can also be an extracellular matrix or extracellular matrix substitute such as Matrigel®. In the invention, the solution of cells comprises a plurality of cells. The cells can be of any cell type. More preferably, the cells are selected from among human, animal and plant eukaryotic cells, even more preferably pluripotent stem cells, progenitors, cells undergoing differentiation and differentiated cells. Where necessary, said pluripotent stem cells can be induced pluripotent stem (IPS) cells, multilineage-differentiating stress enduring (MUSE) cells which are found in the skin and the bone marrow of adult mammals, or embryonic stem (ES) cells. In one particular embodiment, for legal or ethical reasons, the stem cells do not include human embryonic stem cells or cells requiring the destruction of human embryos.

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[0040] In one embodiment of the invention, the solution capable of gelling comprises or consists of a hydrogel, and therefore the outer layer is a three-dimensional structure formed from a matrix of polymer chains swollen by a liquid, and preferably water. For example, the solution capable of gelling comprises or constitutes alginate and preferably, consists of alginate. In the context of the invention, "alginate" is understood to mean linear polysaccharides formed from  $\beta$ -D-mannuronate (M) and  $\alpha$ -L-guluronate (G), salts and derivatives of same. Advantageously, the alginate is a sodium alginate, composed of more than 60%, or more than 80% of G and less than 40%, or less than 20% of M, with an average molecular weight of 100 to 400 KDa and a total concentration of between 0.5% and 5% by weight.

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[0041] In the microcompartments obtained by means of the system according to the invention, the cells present in the inner portion can be isolated and/or in the form of at least one layer and/or in the form of a three-dimensional aggregate and/or in the form of at least one three-dimensional cellular microtissue, optionally with at least one lumen.

[0042] According to one variant, at least one cellular microcompartment obtained by means of the system according to the invention comprises at least one layer of cells and at least one lumen. When the microcompartment comprises at least one lumen, at least one layer of cells, the layer of intermediate solution of the inner portion and the outer layer are preferably, are successively organized around said lumen, a cyst-like configuration is referred to. Thus, according to one variant, at least one cellular microcompartment obtained by means of the system according to the invention comprises at least one cyst, of which the hollow center, or lumen, is preferably aqueous. In the context of the invention, a "cyst" is understood to mean a three-dimensional arrangement of cells in a single layer or an epithelial-based, spherical arrangement surrounding a central lumen. This configuration in the form of cyst(s) makes it possible to reduce the pressures which the cells are subjected to. This configuration also makes it possible to reduce cell death and increase the amplification factor of the culture. Consequently, this makes it possible to reduce the number of passages and dissociation required and to reduce the culturing time required to reach the required number of final cells.

[0043] The cellular microcompartments obtained by means of the system according to the invention preferably comprise one or more cysts, and/or one or more tissues and/or microtissues and/or aggregates of cells with or without lumen(s).

[0044] Advantageously, the system according to the invention is arranged such that each cellular microcompartment obtained by means of this stage system is enclosed. In one embodiment, the system according to the invention is arranged

such that each cellular microcompartment obtained by means of this system is spherical or drop-shaped. Preferably, the diameter of such a microcompartment is between 10  $\mu\text{m}$  and 1 mm, more preferably between 50  $\mu\text{m}$  and 700  $\mu\text{m}$ , even more preferably greater than 200  $\mu\text{m}$ , preferably less than 600  $\mu\text{m}$ .

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[0045] In another embodiment, the system according to the invention can be arranged such that each cellular microcompartment obtained by means of this system has an elongate shape, in particular an ovoid or tubular shape.

10 [0046] Advantageously, each container of the system according to the invention can be a flexible bag, a syringe or a conical-bottom tube. More advantageously, each container is connected to an inlet of the encapsulation device by one or more dispensers, such as ducts, pipes or tubes, a syringe pump or a peristaltic pump allowing the solution contained in the container to be dispensed,  
15 continuously or per dose, to said inlet of the encapsulation device.

[0047] Of course, the various features, variants and embodiments of the invention can be associated with one another according to various combinations insofar as they are not incompatible or exclusive of one another.

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[0048] In addition, various other features of the invention will become apparent from the appended description given with reference to the drawings which illustrate non-limiting embodiments of the invention and wherein:

25 [0049] [Fig. 1] is a perspective view of an embodiment of a fluidic chip according to the invention, the inner channels of which are visible by transparency of the body of the fluidic chip,

[0050] [Fig. 2] is a perspective view of the fluidic chip of Figure 1 comprising a  
30 different viewing angle,

[0051] [Fig. 3] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate A of the central axis Z,

- 5 [0052] [Fig. 4] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate B of the central axis Z,

- 10 [0053] [Fig. 5] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate C of the central axis Z,

- 15 [0054] [Fig. 6] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate D of the central axis Z,

- 20 [0055] [Fig. 7] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate E of the central axis Z,

[0056] [Fig. 8] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate F of the central axis Z,

- 25 [0057] [Fig. 9] is a half-section view of the fluidic chip of Figure 1 at a different viewing angle at the cutting plane passing through the ordinate G of the central axis Z.

- 30 [0058] [Fig. 10] is a view of an embodiment of a cell encapsulation system according to the invention, and

[0059] [Fig. 11] a cross-section view of the encapsulation device of the system of figure 10.

[0060] The dashed line in Figure 1 corresponds to the central axis Z of the fluidic chip.

[0061] The bold lines of Figures 3 to 9 represent the cutting planes at each of the ordinates denoted by A to G on the central axis Z of Figure 1.

[0062] It should be noted that in these figures, the structural and/or functional elements common to the different variants may have the same references.

[0063] The invention aims to stabilize the jet at the outlet of a fluidic chip.

[0064] For these purposes, the invention denoted by the reference 1 in its entirety a fluidic chip consists of a body 2 comprising an upper face 3 and opposite a lower face 4. The upper face 3 comprises a main inlet 5 and at least one secondary inlet 6. The lower face 4 comprises an outlet 7.

[0065] The figures show a fluidic chip 1 comprising a body 2 of circular cylindrical shape about a central axis Z with an upper face 3, a lower face 4 and a lateral face. Other embodiments, such as polygonal, are compatible with the invention. Thus, the faces of the body 2 of the fluidic chip 1 are not necessarily opposite.

[0066] A main channel 8 connects the main inlet 5 to the outlet 7. The main channel 8 is substantially rectilinear. The main inlet 5 is thus aligned with the outlet 7 along the central axis Z shown in Figure 1. According to the embodiment shown, the main channel 8 substantially follows the central axis Z of the fluidic chip 1.

[0067] A fluidic chip 1 according to the invention also comprises a secondary channel 9 that connects the secondary inlet 6 to the outlet 7. A fluidic chip 1 according to the invention may comprise several secondary inlets 6, the

fluidic chip 1 then comprises an equal number of secondary channels 9 which respectively connect each secondary inlet 6 to the outlet 7.

5 [0068] According to the embodiment shown, the main channel 8 and the secondary channel(s) 9 comprise partially circular sections whose dimensions vary and also sections of more complex shape, for example at the separations of a channel in two parts named ducts described below.

10 [0069] Figures 1 and 2 show an embodiment of a fluidic chip 1 comprising a first secondary inlet 6a and a second secondary inlet 6b arranged on either side of the main inlet 5, the three main inlet 5 and secondary inlets 6a, 6b being aligned. According to other embodiments of the invention not shown, a fluidic chip 1 comprises a single secondary inlet 6 or three or more secondary inlets 6. The architecture principle of the secondary channels 9 that are articulated  
15 around the main channel 8 is identical and is explained below. According to other embodiments not shown, the secondary inlets 6 may not be aligned.

[0070] Figure 3 corresponds to a cross-section of Figure 1 at the ordinate denoted A on the central axis Z in Figure 1. The main inlet 5 is thus connected to the main  
20 duct 8. The first secondary inlet 6a is connected to the first secondary duct 9a and the second secondary inlet 6b is connected to the second secondary duct 9b.

[0071] Figure 4 corresponds to a cross-section of Figure 1 at the ordinate denoted B on the central axis Z in Figure 1. The main channel 8 is divided by forming four  
25 rectilinear main ducts 10 parallel to each other. Furthermore, the secondary channels 9a, 9b are each divided, forming two secondary ducts 11a, 11b. The first secondary channel 9a comprises a rectilinear portion so as to come closer to the main channel 8 and then comprises a separation forming an arc of a circle, of diameter D1 relative to the central axis Z. The second secondary channel 9b  
30 comprises a separation forming an arc of a circle, of diameter D2 relative to the central axis Z. Thus D1 is less than D2 so as to optimize the space included in the plane and in particular situated at the ordinate denoted B.

[0072] According to one embodiment not shown, the fluidic chip 1 comprises a third secondary inlet 6 and therefore a third secondary channel 9. Said third secondary channel 9 then comprises a separation forming an arc of a circle of diameter D3 greater than the diameter D2 of the arc of a circle coming from the second secondary channel 9. Depending on the arrangement of the third secondary inlet, the third secondary channel 9 comprises a rectilinear portion making it possible to place the arc of a circle corresponds to the desired diameter D3. This architecture comprising concentric circular arcs is arranged according to the number of secondary inlets 6.

[0073] Advantageously according to the embodiment shown, the separation of each secondary channel 9 takes place at the middle of the arc of a circle in order to form secondary ducts 11 of the same length.

[0074] A secondary channel 9 of a fluidic chip 1 according to the invention therefore comprises at least one rectilinear channel portion substantially parallel to the central axis Z.

[0075] The four ducts 11a, 11b are also visible in Figure 5 which corresponds to a section in Figure 1 at the ordinate denoted C on the central axis Z in Figure 1.

[0076] According to the embodiment shown, the secondary channels 6a, 6b comprise a second division visible in particular in Figure 6 which corresponds to a section in Figure 1 at the ordinate denoted D on the central axis Z in Figure 1. The secondary channels 6a, 6b therefore divide twice, forming four secondary ducts 11a, 11b. This second separation also comprises portions in the shape of an arc of a circle about the central axis Z of the fluidic chip 1 according to the same diameters D1, D2 as for the first separation. The respective circular arcs of the first and second secondary channels 9a, 9b are symmetrical.

[0077] The four secondary ducts 11a, 11b thus formed from each of the secondary channels 9a, 9b are visible in Figure 7 which corresponds to a section in Figure 1 at the ordinate denoted E on the central axis Z in Figure 1. Twelve main 10 and secondary ducts 11a, 11b are thus visible.

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[0078] All the main 8 and secondary 9 channels join at the outlet 7 on a coaxial circular injection duct. According to the embodiment shown and in particular in Figure 8, which corresponds to a section in Figure 1 at the ordinate denoted F on the central axis Z in Figure 1, each main duct 10 and secondary duct 11a, 11b joins respectively on a respective single duct of circular shape. Each main duct 10 and secondary duct 11a, 11b being concentric so that all the ducts join concentrically towards the outlet 7 in the coaxial circular injection duct. Thus, all the jets are coaxial at the outlet of the fluidic chip 1. According to the embodiment shown, the outlet 7 emerges directly into the air. According to other embodiments not shown, the outlet 7 is connected to an accessory or extension such as a nozzle.

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[0079] According to one form of use, a fluidic chip 1 is used for a cell encapsulation system.

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[0080] The cell encapsulation system comprises at least two containers, one of the containers comprising a solution of cells and the other of the containers comprising a solution capable of gelling.

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[0081] The cell encapsulation system also comprises an encapsulation device comprising a fluidic chip 1 according to the invention, the main inlet 5 and secondary inlet 6 each being connected to one of the containers via a dispenser. The fluidic chip 1 is able to form a concentric jet at the outlet from the solutions provided by the dispensers. The encapsulation device is arranged so that the jet divides, at the outlet of the encapsulation device, into drops, of which the outer layer is the solution capable of gelling and the core is the solution of cells.

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[0082] Finally, the cell encapsulation system comprises a gelling bath arranged downstream of the encapsulation device to collect the drops formed by the cell encapsulation device and arranged to cause a gelling of the outer layer of each drop during its immersion in the bath.

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[0083] Figure 10 shows a cell encapsulation system according to one embodiment of the invention.

[0084] The system comprises two containers 110 and 120. A first container 110  
10 comprises a solution comprising a plurality of human pluripotent stem cells. A second container 120 comprises a solution capable of gelling, comprising for example a hydrogel such as an alginate hydrogel. It can be envisaged that the system comprises a third container comprising an intermediate solution, for example comprising an isotonic solution such as sorbitol.

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[0085] The system also comprises an encapsulation device 130 which comprises a fluidic chip 1 and which is arranged to form, from the solutions of containers 110 and 120, cellular microcompartments, the outer layer of which is an alginate solution and the core of which is the solution of cells.

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[0086] Figure 11 shows a cross-section view of the encapsulation device 130.

[0087] As shown in Figure 11, the encapsulation device 130 comprises a plurality of inlets 5,6 each connected to one of the containers 110, 120 via a  
25 dispenser 140. In the example described, each dispenser 140 thereby comprises a dispensing circuit and a movement member, such as a syringe pump or a peristaltic pump, allowing the solution contained in the container 110, 120 to be dispensed, continuously or per dose, to the inlet 5, 6 of the device 130 to which it is connected.

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[0088] It should be noted that the dispenser 140 for dispensing the alginate solution is equipped, in the example described, with a member capable of

electrically charging the alginate solution with electric potential. It can be envisaged, in one variant, that the alginate solution is charged directly in its container 120, via an electrode immersed in this solution.

5 [0089] The encapsulation device 130 comprises a fluidic chip 1. According to this embodiment shown, the fluidic chip 1 comprises a body 2, comprising the inlets 5, 6 and a nozzle 340 connected to a single outlet 7 of the body 2 forming a single outlet of the device 130. It can be envisaged that the body 2 and the nozzle 340 are made of glass or another material suitable for the  
10 pharmaceutical industry. It can be envisaged that the body 2 and the nozzle 340 form a single piece or alternatively that they are produced separately and then assembled to form the encapsulation device 130.

[0090] In the described example, the body 2 comprises a main channel 8  
15 comprising a substantially rectilinear portion defining a central axis of the encapsulation device 130. This main channel 8 connects the main inlet 5 to the single outlet 7 of the body 2. The body 2 comprises a secondary channel 9 connecting the second inlet 6 to the single outlet 7. This secondary channel subdivides into portions extending around the main channel 8, said subdivisions  
20 of the secondary channel 9 joining at the single outlet 7 of the body 2 in a single circular portion which is concentric with the main channel. This single circular portion and the main channel then join to form the single outlet 7 of the body 2.

[0091] In other words, the fluidic chip 1 makes it possible to form a concentric  
25 flow from the solutions provided by the containers 110, 120 via the dispensers 140, an outer flow being formed by the alginate solution and an inner flow being formed by the solution of cells.

[0092] The nozzle 340 thus receives the concentric flow. Taking into account the  
30 flow rate of the solutions, and the electrostatic force generated by the electric charges supported by the alginate solution, the encapsulation device 130 thus generates, at the outlet of the nozzle 340, a concentric jet from the concentric

flow. This concentric jet divides, under the effect of the Plateau-Rayleigh instability, into cellular microcompartments, of which the outer layer is the alginate solution and the core is the solution of cells.

5 [0093] The encapsulation device 130 is therefore an electro-jetting device. It should be noted that the relative sizes of the outer layer and the core of the microcompartments can be adjusted by modifying the ratios of the flow rate of the two solutions using the dispensers 140, while the overall size of the microcompartments can be controlled by adjusting the overall flow rate of the  
10 solutions and the electric potential of the alginate solution.

[0094] Alternatively, it can be envisaged to dimension the flow rates of the solutions as well as the electric potential so that the encapsulation device 130 is an electro-dripping device, and thus form the microcompartments one after  
15 the other directly from the nozzle 340.

[0095] As shown in Figure 11, a metal ring 160, connected to the body by a connector 170, extends downstream of the outlet of the encapsulation device 130 such that the jet or the cellular microcompartments pass through this ring. The  
20 electric field generated by this metal ring makes it possible to assist the dispersion of the cellular microcompartments, in the case of an electro-jetting device.

[0096] Returning to Figure 10, the system further comprises a collection tank 150 arranged underneath the encapsulation device 130, containing a first  
25 solution for collecting cellular microcompartments formed by this encapsulation device 130 and falling due to gravity. To this end, the collection tank 150 comprises a gelling bath.

[0097] The gelling solution comprises a surfactant and a calcium salt making  
30 it possible to crosslink an alginate solution, thereby making it possible to cause stiffening or gelling of the outer layer of each cellular microcompartment during its immersion in the collection tank.

[0098] Each cellular microcompartment obtained by means of this first stage is thereby enclosed and has, in the example described, a spherical shape or the shape of an elongate drop.

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[0099] In one variant, the container comprising the solution of cells and/or the container comprising the intermediate solution comprises at least one extracellular matrix and/or one extracellular matrix substitute. In this case, the encapsulation device 130 can form cellular microcompartments, of which the  
10 outer hydrogel layer comes from the alginate solution, an intermediate layer forming a cellular matrix and/or an extracellular matrix substitute from the solution of cells and/or the intermediate solution and at least one layer and/or base and/or aggregate and/or cyst of cells from the solution of cells. In this example, it can be envisaged that the solution electrically charged with said electric potential is the  
15 alginate solution and/or the intermediate solution of sorbitol.

[0100] It can be noted that the collection tank 150 comprises an outlet 1510 for extracting the gelling solution and the microcompartments immersed in this solution as well as an inlet 1520 for injecting the gelling solution into the collection  
20 tank 150. This inlet 1520 and outlet 1510 are connected to a collection circuit.

[0101] In the example described, the collection tank is formed by a lower portion 1530 containing the gelling solution and on which the extraction outlet 1510 and injection inlet 1520 are provided and an upper portion 1540 forming  
25 a cover on which the encapsulation device 130 is mounted and the metal ring 160. The collection tank 1530 – cover 1540 assembly thereby defines an enclosed chamber, which is appropriate for the production of cellular microcompartments with regard to the necessary sterility requirements.

[0102] Of course, various other modifications can be made to the invention  
30 within the scope of the appended claims.

## Claims

1. A fluidic chip (1) composed of a body (2) comprising a main inlet (5) and at least one secondary inlet (6) not coaxial to the main inlet (5), each  
5 main inlet (5) and secondary inlet (6) being intended to receive a substance and an outlet (7), the main inlet (5) is connected to the outlet (7) by a main channel (8) comprising a substantially rectilinear portion defining a central axis Z for the fluidic chip (1), the secondary inlet is also connected to the outlet (7) by a secondary channel (9), characterized in that the secondary  
10 channel (9) is divided into at least two secondary ducts (11) of the same dimensions which join in a circular injection duct coaxial with the main channel (8) in an evenly distributed manner about the central axis Z.
2. The fluidic chip (1) according to the preceding claim, obtained by a  
15 subtractive printing technique on glass.
3. The fluidic chip (1) according to one of the preceding claims, wherein the body (2) comprises an upper face (3) comprising the main inlet (5) and at least the secondary inlet (6) and a lower face (4), opposite the upper face (3),  
20 comprising the outlet (7).
4. The fluidic chip (1) according to one of the preceding claims, wherein the geometry of the main channel (5) and secondary channel (6) is three-dimensional.  
25
5. The fluidic chip (1) according to one of the preceding claims, wherein the geometry of the fluidic chip (1) is compact along the outlet axis.
6. The fluidic chip (1) according to one of the preceding claims, wherein the  
30 secondary channel (9) comprises a first separation at an ordinate B of the central axis Z and a second separation at an ordinate D of the central axis Z, thus defining four secondary ducts (11) of the same length.

7. The fluidic chip (1) according to one of the preceding claims, wherein the secondary channel (9) comprises at least one rectilinear portion opening at the center of a portion in the shape of an arc of a circle extending in a plane perpendicular to the central axis Z.
8. The fluidic chip (1) according to any one of the preceding claims, comprising a first and a second secondary inlet (6a, 6b) connected respectively to a first and a second secondary channel (9a, 9b), the first secondary channel (9a) comprising a portion in the shape of an arc of a circle of diameter D1 relative to the central axis Z extending in a plane perpendicular to the central axis Z, and the second secondary channel (9b) comprising a portion in the form of an arc of a circle of diameter D2 relative to the central axis Z extending in a plane perpendicular to the central axis Z, with the diameter D1 being less than the diameter D2.
9. The fluidic chip (1) according to the preceding claim, wherein the first and the second secondary channel (9a, 9b) each comprise two circular arc-shaped portions at two different ordinates of the central axis Z each therefore defining four secondary ducts (11a, 11b) of the same length.
10. The fluidic chip (1) according to one of the preceding claims, wherein the main channel (8) comprises four main ducts (10) of the same length.
11. The fluidic chip (1) according to one of the preceding claims, wherein each main channel (8) and secondary channel (9) comprises a circle-shaped portion opening onto the outlet (7), each circle-shaped portion being concentric.
12. A cell encapsulation system, the system comprising at least:  
- two containers (110, 120), one of the containers comprising a solution of cells and the other of the containers comprising a solution capable of gelling,

- an encapsulation device (130) comprising a fluidic chip (1) according to one of claims 1 to 11, the main inlet (5) and secondary inlet (6) each being connected to one of the containers (110, 120) via a dispenser (140) and able to form a concentric jet from the solutions provided by the dispensers (140),  
5 the encapsulation device (130) being arranged so that the jet divides, at the outlet of the encapsulation device (130), into drops, of which the outer layer is the solution capable of gelling and the core is the solution of cells, and
- a gelling bath arranged downstream of the encapsulation device (130) to collect the drops formed by the encapsulation device (130) and arranged to  
10 cause a gelling of the outer layer of each drop during its immersion in the bath.