

BIOCOMPATIBLE COMPOSITION SUITABLE FOR ADMINISTERING CELL THERAPIES AND USE THEREOF IN PARTICULAR PARKINSON'S DISEASE

5 TECHNICAL FIELD

[0001] The present invention relates to the treatment of Parkinson's disease. In particular, the invention relates to a specific biocompatible composition comprising a solution and heterogeneous cellular elements suspended in
10 said solution.

BACKGROUND ART

[0002] Parkinson's disease is a neurodegenerative disorder characterized by
15 a progressive decrease in dopamine production in the brain. This decrease in dopamine leads to motor symptoms such as slowness in initiating movements (akinesia), muscle rigidity, and resting tremor.

[0003] Currently, one of the most promising treatments for Parkinson's disease is
20 the injection of dopaminergic neurons into the putamen, a region of the brain involved in motor control. This approach, known as cell therapy, aims to restore impaired dopaminergic function by providing functional cells capable of producing and releasing dopamine. Cell therapy by injection of dopaminergic neurons offers numerous benefits, including long-lasting symptom relief, reduced medication
25 use, and improved quality of life for patients with Parkinson's disease.

[0004] Today, cell therapy faces numerous challenges inherent in the administration of cells into the body.

30 [0005] In addition to the difficulties involved in large-scale cultivation of therapeutic cells tailored to the target organ, the composition of the injection

medium containing the cells to be inoculated is a fundamental challenge for any injectable therapy, particularly when administered to the brain.

5 [0006] In general, drugs may be dissolved in a liquid and administered as a solution, such as through an IV drip. Liquid suspensions containing very small particles also behave like solutions and can also be administered by infusion.

10 [0007] Conversely, once the composition is cellularized, that is to say, when it comprises cells, it is injected as a suspension.

[0008] Unlike solutions that form a homogeneous, free-flowing liquid, suspended cells naturally sediment over time.

15 [0009] For example, “normal” red blood cells sediment in blood at a relative rate of up to 20 mm per hour, and when certain cells are aggregated into clusters or microtissues, this relative rate may exceed 100 mm per hour, or even 300 mm per hour in some cases.

20 [0010] Particle sedimentation in solid-liquid suspensions is a subject that has been widely discussed in numerous fields, such as chemistry, geology, and biology, particularly in pharmaceutical applications and bioprinting.

25 [0011] The sedimentation rate of solid particles, such as cells, in a liquid refers to the rate at which the particles move downward or upward or settle on the bottom of a container due to gravity. This sedimentation phenomenon occurs when the particles, due to their mass and density, are heavier or lighter than the medium in which they are suspended.

30 [0012] When a cell suspension is used, the cells may be heterogeneous. Indeed, the cells may be present in a wide variety of forms, in which case they are referred to as cellular elements. By way of example, the cellular elements may be in the form of isolated cells, cellular microtissues, or cell

clusters having different density and/or size and/or cell count. Consequently, when suspensions containing heterogeneous cellular elements are used, the cells are distributed heterogeneously within their medium, as a function of several parameters, such as the size, shape, and density of the cells, as well
5 as the physicochemical properties of the medium.

[0013] Without control of these parameters, the highest concentration of cells is found, due to gravity, toward the bottom of the device containing the suspension of cellular elements.

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[0014] By way of example, in cell therapy, during injection of a suspension containing heterogeneous cellular elements, the first injection volume may contain many more cells than those distributed subsequently. This density gradient creates heterogeneity within the different injection volumes, which
15 may compromise the effectiveness of the cell therapy.

[0015] In the context of the treatment of Parkinson's disease, cell therapy consists in directly injecting cells, preferentially functional dopaminergic neurons, into the putamen. The success of such a technique depends heavily
20 on the ability to precisely control the amount of cellular elements injected. Indeed, it is necessary to inject an equivalent amount of cells into several regions of the putamen precisely defined during the surgical procedure.

[0016] Furthermore, beyond maintaining a homogeneous distribution of cells
25 within their medium, maintaining biological stability is essential to ensuring the survival of living cells in their medium, particularly when injecting viable cells for use in cell therapy. Indeed, the osmolality, concentration, and diversity of the ionic species are essential for maintaining the homeostasis of cells in suspension.

[0017] There is therefore a significant need for a biocompatible composition, comprising heterogeneous cellular elements, which is capable of maintaining:

- the homogeneous distribution of cellular elements within the composition, so as to enable precise control of the amount of cells contained in a given volume; and
- the biological stability of the cellular elements in suspension.

5 SUMMARY OF THE INVENTION

[0018] To address this need, the invention proposes a novel biocompatible composition comprising a solution and cellular elements suspended in said solution, said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and mixtures thereof, at least two of said cellular elements having different density and/or size and/or cell count, said solution comprising at least:

- hyaluronic acid and/or carboxymethyl cellulose (CMC), and
- at least one biocompatible density agent,
- and/or at least one modified form of one or more of these molecules

[0019] Hyaluronic acid and CMC are biocompatible viscosity agents that are particularly well-suited to the context of the invention.

[0020] Indeed, hyaluronic acid is a substance that occurs naturally in the human body, primarily in the skin, joints, and connective tissues. It consists of a chain of sugars and plays an essential role in maintaining skin hydration, elasticity, and firmness.

[0021] Sodium hyaluronate, a soluble form of hyaluronic acid, is widely used in aesthetic and regenerative medicine, particularly to increase the volume and shape of certain parts of the body, such as the lips, cheeks, or deep wrinkles. Sodium hyaluronate is a biocompatible molecule, which considerably reduces the risks of adverse effects and intolerance.

[0022] Carboxymethyl cellulose (CMC) is a biocompatible and biodegradable polymer derived from cellulose.

[0023] According to a preferred embodiment, the composition according to the invention has a static viscosity, measured at 25°C, of between 0.01 and 1.5 Pa·s, preferentially of between 0.01 and 1 Pa·s, more particularly of
5 between 400 and 1200 mPa·s, more preferentially of between 400 and 1000 mPa·s, and even more preferentially of between 500 and 1100 mPa·s, in particular of between 500 and 900 mPa·s.

[0024] According to a preferred embodiment, the composition according to
10 the invention comprises dextran as a density agent.

[0025] Dextran is a biocompatible, water-soluble polymer of glucose derived from starch and may be produced from plant or microbial sources.

15 [0026] Preferably, the composition according to the invention has a density of between 800 and 2000 kg/m³, preferentially of between 997 and 1600 kg/m³, preferentially of between 1025 and 1100 kg/m³, in particular of between 1047 and 1090 kg/m³, and most preferentially of between 1050 and 1090 kg/m³.

20 [0027] The rheological characteristics of the composition according to the invention make it possible to use the composition according to the invention in injection devices without the fluid friction or shear forces encountered during injection impairing the precision of the injection or impairing the viability and integrity of the cells. Indeed, an excessive viscosity and/or density may result in
25 increased resistance as the composition flows through the injection means, combined with viscous friction forces that may damage the cellular elements.

[0028] The inventors have succeeded in developing a biocompatible composition having rheological characteristics suited to heterogeneous
30 cellular elements, based on the density/viscosity combination of the solution comprising said cellular elements.

[0029] Furthermore, hyaluronic acid, CMC, and dextran are biocompatible and are particularly well-suited to the context of the invention. Indeed, these molecules modify the rheological characteristics of the composition according to the invention in such a way as to reduce the maximum sedimentation rate of the heterogeneous cellular elements present in the solution, thereby ensuring a uniform distribution of the cellular elements within the solution.

[0030] According to a preferred aspect of the invention, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the cellular elements have a maximum sedimentation rate of less than $20 \text{ mm} \cdot \text{h}^{-1}$ with respect to the solution.

[0031] The maximum sedimentation rate of less than $20 \text{ mm} \cdot \text{h}^{-1}$ of the cellular elements with respect to the solution provides numerous advantages to the composition according to the invention, namely:

- increased cell viability: A maximum sedimentation rate of less than $20 \text{ mm} \cdot \text{h}^{-1}$ reduces the mechanical stress exerted on the cellular elements, thereby minimizing the risk of alteration to their structure or metabolism, which may improve their viability;
- increased analytical precision: In the context of specific analyses and measurements, a maximum sedimentation rate of less than $20 \text{ mm} \cdot \text{h}^{-1}$ ensures greater precision and sensitivity of the results;
- improved homogeneity: A maximum sedimentation rate of less than $20 \text{ mm} \cdot \text{h}^{-1}$ ensures that the cells remain homogeneously distributed throughout the solution, thereby preventing the formation of concentration gradients;
- improved experimental reproducibility: By maintaining a maximum sedimentation rate of less than $20 \text{ mm} \cdot \text{h}^{-1}$, the variations due to differential sedimentation of the heterogeneous cellular elements are reduced.

[0032] According to a preferred aspect, the composition according to the invention comprises a solution and cellular elements, wherein the osmolality

of the cellular elements in the solution is between 180 and 360 mOsmol/kg, preferentially between 220 and 360 mOsmol·kg⁻¹.

5 [0033] Advantageously, the osmolality of the cellular elements helps create an environment that is conducive to their survival, such as an isotonic environment.

10 [0034] Surprisingly, the inventors have developed a biocompatible composition suitable for a numerous applications, which controls the sedimentation rate of the heterogeneous cellular elements suspended in the solution while exhibiting biological stability suitable for cells, particularly human or animal cells.

15 [0035] According to a preferred aspect of the invention, the composition comprises a solution and cellular elements suspended in said solution, wherein the cells constituting the cellular elements are selected from neural cells, neuronal cells, glial cells, and mixtures thereof.

20 [0036] Preferentially, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein at least one cell, preferentially a majority of the cells, constituting the cellular elements is/are dopaminergic neuron(s).

25 [0037] According to another aspect, the invention relates to the biocompatible composition according to the invention for use in the treatment of Parkinson's disease.

[0038] Preferentially, the biocompatible composition according to the invention is used as an injectable composition in humans or animals.

30 [0039] Advantageously, by ensuring a uniform distribution of the cellular elements within the solution, the composition according to the invention makes

it possible to obtain precise dosing of the injected cellular elements, thereby improving:

- therapeutic efficacy: When cellular elements are injected for therapeutic purposes, precise dosing is essential to achieve the desired result. An
5 insufficient amount of cellular elements may not be effective, while an overdose of cellular elements may lead to adverse effects. By precisely adjusting the dose, it is possible to maximize therapeutic efficacy while minimizing risks to the patient;
- experimental precision: Controlling the dose during injection of cellular
10 elements makes it possible to minimize variations between samples and to obtain reliable and reproducible data. This is particularly important in comparative experiments or when testing the efficacy of new cell therapies.
- resource optimization: The resources used for the production of cellular elements, such as cell cultures and culture media, may be costly. Accurate
15 dosing makes it possible to minimize waste of these resources by avoiding unnecessary overdosing.

[0040] The invention also relates to a method for preparing a biocompatible composition according to the invention, which comprises carrying out the
20 following steps:

- (a) Preparing or recovering a composition comprising a set of cellular elements, at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 μm ;
- 25 (b) Preparing a solution comprising:
 - hyaluronic acid and/or carboxymethyl cellulose (CMC), and
 - at least one biocompatible density agent,
 - and/or at least one modified form of one or more of these molecules;
- (c) Sterilizing the solution resulting from step (b) so as to obtain a sterilized
30 filtrate;
- (d) Mixing the cellular elements resulting from step (a) with the sterilized filtrate resulting from step (c);

(g) Optionally, recovering a biocompatible composition comprising a solution and mesoscopic objects suspended in said solution, at least two of said mesoscopic objects having a different density and/or size; preferentially at least one cellular element is a microtissue having a largest dimension of at least 50 μm , and said composition comprises the following molecules:

- Hyaluronic acid and/or carboxymethyl cellulose (CMC);
- At least one biocompatible density agent;
- and/or at least one modified form of one or more of these molecules.

[0041] Advantageously, the method according to the invention makes it possible to prepare a biocompatible composition having a combination of density and viscosity that is optimal for maintaining the heterogeneous cellular elements in suspension with a maximum sedimentation rate with respect to the solution of less than 20 $\text{mm}\cdot\text{h}^{-1}$.

[0042] Finally, according to a last aspect, the invention relates to a kit comprising:
* a biocompatible composition comprising a solution and cellular elements suspended in said solution, said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and mixtures thereof, at least two of said cellular elements having different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 μm , said composition comprising at least:

- hyaluronic acid and/or carboxymethyl cellulose (CMC), and
- at least one biocompatible density agent,

- and/or at least one modified form of one or more of these molecules
and

- * an injection means, preferentially a cannula.

[0043] Further features and advantages will become apparent from the detailed description of the invention, the examples, and the figures that follow.

BRIEF DESCRIPTION OF THE FIGURES:

[0044] [Figure 1] Figure 1 depicts a sequence of images illustrating the stability of cellular elements in suspension over time in a composition according to the invention, and a comparative composition.

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[0045] [Figure 2] Figure 2 depicts a comparative study of the impact of prolonged immersion (6 hours) of cellular elements in a solution, on a panel of production batches of the microtissue of interest.

10 [0046] [Figure 3] Figure 3 is a schematic depiction of a comparative study evaluating the impact of an injection procedure on the viability of the cellular elements within the composition according to the invention.

[0047] [Figure 4] Figure 4 is a graphical depiction of a study evaluating the various shear deformations of the composition according to the invention.

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[0048] [Figure 5] Figure 5 is a graphical depiction of a comparative study of the impact of prolonged immersion (12 hours) of cellular elements in different solutions.

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DESCRIPTION OF THE INVENTION

Definitions:

25 [0049] For the purpose of the invention, the term “density agent” refers to a biocompatible chemical species that is soluble in an aqueous solution and is used to increase the density of the solution without substantially altering the biocompatibility of the solution with the suspended cellular elements. More specifically, when a density agent as defined by the invention is added to an

30 aqueous solution at a concentration greater than 10% by mass, this does not lead to any change in the rheological behavior of the solution. Furthermore, adding such an agent does not increase the osmolality of the solution beyond

360 mOsm. In other words, adding a density agent as defined by the invention at a concentration greater than 10% by mass to a solution does not cause a phase transition such as gelation.

- 5 [0050] For the purpose of the invention, the term “cell cluster” refers to a three-dimensional grouping of cells held together by adhesive interactions or by the presence of interstitial tissue.

- 10 [0051] For the purpose of the invention, the term “biocompatible” refers to the ability of a substance or composition to interact favorably with biological systems, such as tissues, organs, or living organisms, without causing harmful reactions, excessive irritation, or undesirable damage. When a substance or composition is described as biocompatible, this means that it is designed and formulated to minimize the risk of toxicity, inflammation, rejection, or other
15 adverse reactions from the body and/or the cellular elements.

- [0052] For the purpose of the invention, “human cells” refers to human cells or immunologically humanized non-human mammalian cells. Even where not specified, the cells, the stem cells, the progenitor cells and the tissues
20 according to the invention are constituted by or are obtained from human cells or from immunologically humanized non-human mammalian cells.

- [0053] For the purpose of the invention, “progenitor cell” refers to a stem cell already engaged in cellular differentiation but not yet differentiated.
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- [0054] For the purpose of the invention, the term “embryonic stem cell” refers to a pluripotent stem cell derived from the inner cell mass of a blastocyst. The pluripotency of embryonic stem cells may be assessed by the presence of markers such as the transcription factors OCT4, NANOG, and SOX2, and
30 surface markers such as SSEA4/5, Tra-1-60, and Tra-1-81. The embryonic stem cells used in the context of the invention are obtained without destroying the embryo from which they are derived, for example using the technique

described in Chang et al. (Cell Stem Cell, 2008, 2(2)): 113–117). Optionally, human embryonic stem cells may be excluded.

5 [0055] For the purpose of the invention, “pluripotent stem cell” or “pluripotent cell” refers to a cell which has the ability to form all the tissues present in the entire organism of origin, without however being able to form an entire organism as such. Human pluripotent stem cells may be referred to as hPSCs in the context of the present invention. In particular, these may be induced pluripotent stem cells (iPSC or hiPSC for human induced pluripotent stem cells), embryonic
10 stem cells or MUSE cells (for “Multilineage-differentiating Stress Enduring”).

[0056] For the purpose of the invention, “induced pluripotent stem cell” refers to a pluripotent stem cell induced to pluripotency by genetic reprogramming of differentiated somatic cells. These cells are notably positive for
15 pluripotency markers such as alkaline phosphatase staining and expression of NANOG, SOX2, OCT4 and SSEA4/5 proteins. Examples of methods for obtaining induced pluripotent stem cells are described in the articles by Yu et al. (Science 2007, 318 (5858): 1917-1920), Takahashi et al (Cell, 207, 131(5): 861-872) and Nakagawa et al (Nat Biotechnol, 2008, 26(1): 101-106).

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[0057] For the purpose of the invention, “cell layer” or “cell bed” refers to several cells forming a layer or a bed that can be structured around a lumen; it may for example be a tissue or a cellular micro-tissue or a three-dimensionally grouped culture. The thickness of the cell layer can be variable.
25 This layer is organized in three dimensions in the microcompartment.

[0058] For the purpose of the invention, the term “rheological behavior” refers to the response of a material to the stresses and strains applied to it. It describes how matter flows or deforms under the influence of an external
30 force. There are several types of rheological behavior, such as elasticity, plasticity, viscosity, and viscoelasticity.

[0059] For the purpose of the invention, the term “density” refers to the density of the entities in question (cellular elements or solutions), expressed in mg/mL. In the context of the invention, the density of the solutions is measured at a temperature of 20°C using a densitometer. In the case of the

5 cellular elements, density is measured by the density gradient method. This method consists in preparing solutions that are density-calibrated and isotonic. These solutions of known density are then introduced in successive layers into a tube so as to form a density gradient from bottom to top, with each layer being denser than the layer directly above it. Typically, density

10 gradients are prepared using density increments of 5 kg/m³. The density of the cellular elements is therefore given with a resolution of +/- 5 kg/m³. Once the density gradient has been formed, the cellular elements are injected into the upper portion of the tube. These particles sediment and stabilize within or between the layer or layers whose density is closest to their own. The

15 reading is taken once all cellular elements have stabilized, typically after 1 hour. Advantageously, colored synthetic beads having calibrated densities may be introduced simultaneously, their equilibrium position providing a positive control for measuring the position of the cellular elements. All of these measurements are taken at the operating temperature or within the

20 projected operating temperature range, since temperature may differentially affect the density of water, the solutions, and the cellular elements.

[0060] For the purpose of the invention, the term “average density of the cellular elements” refers to the average density of cellular elements such as

25 microcompartments. In the context of the invention, the average density of the cellular elements is measured by the density gradient method. This method, which is well known to the skilled person, consists in preparing calibrated solutions of known density. These solutions are then introduced into a tube so as to form a density gradient from bottom to top, each layer

30 being denser than the layer immediately above it. Once the density gradient has been formed in the tube, the cellular elements are introduced into said tube and sediment, stabilizing within or between the layers whose density is

closest to their own. The reading is taken once all the cellular elements have stabilized, typically after 1 hour. Simultaneously with the injection of cellular elements, colored synthetic beads of known density are also introduced into the tube so as to provide a positive control. The average density is then
5 calculated based on the distribution of the cellular elements within said tube.

[0061] For the purpose of the invention, the term “Ferret diameter” of a cellular element refers to the distance between two parallel tangents, such that the entire cellular element lies between these two parallel tangents.
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[0062] For the purpose of the invention, “differentiated” cells refers to cells that display a particular phenotype, as opposed to pluripotent stem cells that are undifferentiated or progenitor cells that are in the process of differentiation.

15 [0063] For the purpose of the invention, the term “cellular element” refers to a component comprising at least one human, animal, or plant cell and taking various forms, such as isolated cells, cellular microtissues, cell clusters, encapsulated cells, encapsulated microtissues, cell clusters (whether encapsulated or not), and mixtures thereof. Microtissues and/or clusters,
20 whether encapsulated or not, may contain one or more lumens.

[0064] For the purpose of the invention, the term “modified forms of these molecules” means that the molecules have been modified by adding one or more molecular groups to one or more functional groups present on the
25 molecules. By way of example, hyaluronic acid consists of an assembly of monomers, each monomer comprising hydroxyl, carboxyl, and N-acetyl functional groups capable of serving as attachment sites for molecular groups. The hydroxyl and carboxyl groups are preferred sites for the attachment of lipids, polymers, or other active molecules to the hyaluronic acid monomer. In
30 addition, amine groups may also be added to the N-acetyl functional group. This makes it possible to create hyaluronic acid esters, hyaluronic acid dialdehydes, N-deacetylated hyaluronic acid, or Cys-hyaluronic acid. Similarly,

dextran, which also consists of monomers, contains hydroxyl functional groups that are accessible to a wide variety of addition reactions (hydroxyl group chemistry).

5 [0065] For the purpose of the invention, “largest dimension” refers to the value of the largest Feret diameter. The size of the cellular elements may be determined by various techniques. In particular, it may be characterized using a Coulter counter. Alternatively, it may be determined optically by microscopy.

10 [0066] For the purpose of the invention, the term “maintaining the cellular elements in suspension” refers to slowing the sedimentation rate of the cellular elements without impairing their survival.

[0067] For the purpose of the invention, “microcompartment” or “capsule” also
15 refers to a partially or totally enclosed three-dimensional structure, containing one or more cellular elements. The structure preferentially consists of a rigidified outer hydrogel layer and a hollow inner portion comprising at least one cellular element and, optionally, other elements such as an extracellular matrix or an extracellular matrix substitute.

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[0068] For the purpose of the invention, the term “microtissue” refers to a community of cells exhibiting cellular interactions, optionally mechanical cohesion, and at least partially replicating a cellular composition present in human tissue under physiological or pathological conditions. Typically, a
25 microtissue exhibits a specific topology in the distribution of its constituent cells. For the purpose of the invention, a microtissue has a largest dimension of at least 50 μm , more preferentially of between 50 μm and 1.3 mm, and in particular of between 100 μm and 800 μm .

30 [0069] For the purpose of the invention, the term “osmolality of cellular elements” refers to a measurement of the total concentration of particles dissolved within the cellular elements, expressed in osmoles per kilogram of water (Osmol/kg H_2O).

[0070] It encompasses active solutes, such as electrolytes and non-ionic molecules. In the context of the invention, osmolality may be measured using a freezing-point osmometer of the “Osmotech XT” type. It should be noted
5 that the use of different models of freezing-point osmometers may render the measurement impossible. Indeed, the composition of the solutions and their viscosity make measuring osmolality a complex task and require high-powered instruments. Alternatively, osmolality may be calculated by extrapolation based on a measurement of a dilution of the solution, which
10 allows for the use of standard instruments.

[0071] For the purpose of the invention, the term “active ingredient” refers to one or more substances responsible for a pharmacological or therapeutic activity.

15 [0072] For the purpose of the invention, “biological stability” refers to the maintenance of the integrity of the cellular elements.

[0073] For the purpose of the invention, “tissue” or “biological tissue” refers to the common meaning of tissue in biology, i.e. the intermediate level of
20 organization between the cell and the organ. A tissue is a set of similar cells of the same origin (usually from a common cell lineage, although they may originate from an association of distinct cell lineages), grouped together in a cluster, network or bundle (fiber). A tissue forms a functional whole, i.e. its cells work together to perform the same function. Biological tissues
25 regenerate regularly and are joined together to form organs.

[0074] For the purpose of the invention, the expression “sedimentation rate threshold” or “maximum sedimentation rate” refers to the maximum measured relative rate of vertical movement within the solution relative to the reference
30 frame of the system, container, or fluidic element in which the suspension is contained. The maximum relative rate is expressed in mm/h. By convention, the relative rate is defined as positive when the cellular elements sediment in

the solution, and negative when they rise within the solution. The term “maximum” refers to the absolute value of the rate, regardless of its sign, in the sense that it is important in the context of the invention for the cellular elements to remain in suspension, which implies that their relative rate should
5 be as low as possible, in absolute value. In the context of the invention, the sedimentation rate may be measured by image analysis, preferentially at room temperature, and even more preferentially between 17 and 23°C.

[0075] For the purpose of the invention, the term “viscosity” refers to the dynamic
10 viscosity that characterizes the resistance of a fluid to laminar flow. It is expressed in Pa·s. In the case of non-Newtonian fluids, this viscosity may depend on the shear stresses applied to the fluid. In particular, the term “shear-thinning fluid” refers to a fluid in which the viscosity decreases with the shear rate. Similarly, a
15 fluid is referred to as a “threshold fluid” if its viscosity is very high at low shear rates but drops sharply beyond a threshold shear rate value. These two categories of fluids are advantageous in the context of the invention because their high static viscosity prevents sedimentation, whereas the fluidization at high shear rates facilitates the flow of the solution and limits viscous frictional forces on the biological elements, for example during injection. In the context of the invention,
20 viscosity may be measured at 25°C using a rheometer via the shear-viscosity curve method, with the fluid confined within a 25-mm-diameter cone-and-plate geometry, having an angle of 1°, with a sandblasted surface, and over a range of shear rates from $\dot{\gamma} = 0.1 \text{ s}^{-1}$ to $\dot{\gamma} = 1000 \text{ s}^{-1}$.

25 [0076] “Static viscosity” refers to the dynamic viscosity measured at rest, that is, at a very low shear rate $\dot{\gamma}$ on the fluid, typically $\dot{\gamma} = 0.1 \text{ s}^{-1}$.

[0077] For the purpose of the invention, the term “viability” refers to the proportion of healthy cells in a sample of cellular elements. In the context of the
30 invention, the proportion of healthy cells is assessed on the basis of the metabolic activity of a cell sample. Metabolic activity is quantified by measuring the ATP concentration in a suspension of lysed cellular elements. The technique

used is commercially available under the name “CellTiter-Glo® 3D Cell Viability Assay”. It is specifically designed to determine cell viability in 3D microtissue spheroids. The assay reagent penetrates large spheroids and has increased lytic capacity, allowing for a more precise determination of viability compared to
5 other assay methods. Preferentially, viability is expressed as a percentage with respect to a control condition. In this case, the viability is said to be “relative”. For example, if condition A is taken as the reference, a viability of 85% for condition B represents a 15% decrease in viability with respect to condition A.

10 Biocompatible composition according to the invention

[0078] The present invention relates to a biocompatible composition comprising a solution and cellular elements suspended in said solution, said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and
15 mixtures thereof, at least two of said cellular elements having different density and/or size and/or cell count, said solution comprising the following molecules:
- hyaluronic acid and/or carboxymethyl cellulose (CMC);
- dextran; and/or
- a salt of one or more of these molecules.

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[0079] Surprisingly, the inventors have developed a biocompatible composition capable of maintaining heterogeneous cellular elements in suspension while maintaining their biological stability over a long period of time. Thus, the choice of compounds and the rheological characteristics of the solution advantageously
25 make it possible to maintain a homogeneous distribution of the cellular elements within the composition without compromising the viability of the cellular elements.

[0080] According to one particular embodiment of the invention, the composition comprises at least one encapsulated cellular element, preferentially contained
30 in a three-dimensional microcompartment comprising an outer hydrogel layer. The microcompartment may be a solid hydrogel sphere. Preferentially, the composition comprises at least one encapsulated cellular element, preferentially

contained in a hollow three-dimensional microcompartment comprising an outer hydrogel layer and a hollow inner portion containing the encapsulated cellular element(s). The hollow inner portion may also contain extracellular matrix and/or one or more extracellular matrix substitutes and/or a solution and/or other elements. According to one particular embodiment, the outer hydrogel layer of the microcompartment may comprise, or consist exclusively of, alginate. The alginate may in particular be a sodium alginate, composed of 80% α -L-guluronate and 20% β -D-mannuronate, having a Young's modulus greater than 10 kPa, preferentially greater than 60 kPa, more preferentially greater than 100 kPa. According to one embodiment, the outer hydrogel layer comprises alginate, said alginate having an average molecular weight of 100 to 400 kDa, more preferentially a molecular weight between 150 and 250 kDa. When the hydrogel of the outer layer of the microcompartment is alginate, the concentration of the alginate solution intended to form said outer layer of the microcompartment is preferentially between 0.5 and 5% by mass, more preferentially the concentration is equal to 2% (plus or minus 0.5%) by mass.

[0081] Preferably, the composition according to the invention comprises a solution and cellular elements, whether encapsulated or not, suspended in said solution, wherein the cells constituting the cellular elements are mature cells and/or progenitor cells and/or stem cells.

[0082] Preferentially, the composition comprises a solution and cellular elements suspended in said solution, wherein the cells constituting the cellular elements are selected from neural cells, neuronal cells, glial cells, and mixtures thereof.

[0083] When the composition according to the invention comprises cellular elements, wherein the cells constituting the cellular elements are glial cells, these may be selected from astrocytic cells and/or oligodendrocytic cells.

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[0084] Preferentially, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein at least one cell constituting the cellular elements is a dopaminergic neuron.

- 5 [0085] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein a majority of the cells constituting the cellular elements are dopaminergic neurons.

[0086] According to one embodiment, the composition according to the
10 invention comprises cellular elements comprising astrocytic cells, oligodendrocytic cells, and dopaminergic neurons.

[0087] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein
15 the cells constituting the cellular elements belong to at least two distinct cell types.

[0088] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein said cellular elements are in the form of microtissues
20 comprising dopaminergic neurons and, optionally, astrocytic cells.

[0089] Advantageously, the cellular elements present in the composition according to the invention are heterogeneous. In particular, they may be in the form of microtissues, whether encapsulated or not, comprising several
25 different cell types that replicate the tissue architecture and cellular interactions necessary to promote tissue regeneration and functionality.

[0090] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said
30 solution, wherein at least 20%, preferentially at least 30%, and in particular at least 40% of the cellular elements are in the form of a microtissue.

[0091] According to one embodiment, the composition according to the invention comprises between 10 and 100% of cellular elements in the form of a microtissue.

5 [0092] According to another embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, in which less than 15%, preferentially less than 10%, and in particular less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of the cellular elements, by number, are in the form of isolated cells.

10 [0093] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein none of the cellular elements are in the form of an isolated cell.

15 [0094] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein at least one cellular element is a microtissue having, preferentially, a largest dimension of at least 50 μm , more preferentially of between 50 μm and 1.3 mm, in particular of between 100 μm and 800 μm .

20 [0095] According to this embodiment, the biocompatible composition according to the invention comprises at least one microtissue in which the largest dimension is at least 50 μm . Unexpectedly, the inventors have succeeded in maintaining the cellular elements, including at least one microtissue, in suspension in a liquid composition. This characteristic makes the composition easy to inject.

25 [0096] In the context of the invention, the composition according to the invention is in liquid form. More specifically, the composition according to the invention is in liquid form and comprises a liquid solution and cellular elements suspended in said solution.

30 [0097] Furthermore, it is advantageous for the biocompatible composition not to be in gel form.

[0098] In the context of the invention, the percentage of cellular elements in the form of microtissues or isolated cells is expressed as the cumulative volume of cellular elements.

5

[0099] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the volume fraction of the cellular elements is between 0.01 and 80% by volume, preferentially between 0.01 and 60%, in particular
10 between 0.01 and 50%, more preferentially between 0.01 and 45%, and even more preferentially between 15 and 30%. According to another embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the cellular elements have a largest dimension of less than 1.3 mm, preferentially of less than 1 mm, in
15 particular of less than 700 μm , and most preferentially of less than 500 μm .

[0100] Preferentially, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the cellular elements have a largest dimension of between 50 μm and 1.3 mm,
20 preferentially of between 100 μm and 800 μm .

[0101] In other words, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the cellular element having the largest dimension has a largest dimension of less
25 than 1.3 mm, preferentially less than 700 μm , preferably less than 500 μm .

[0102] According to one particular embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the composition comprises between 1 million and 500 million cells per
30 mL of solution, preferentially at least 5 million cells, in particular 50 million cells.

[0103] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the composition comprises between 1 and 100 million cells per mL of solution, preferentially between 1 and 50 million cells, more particularly between 1 and 10 million cells, more preferentially between 2 and 6 million cells, and even more preferentially between 3 and 5 million cells per mL of solution.

[0104] Preferentially, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the composition comprises between 500 and 10,000 dopaminergic neurons per μL of solution, in particular at least 1,000 dopaminergic neurons per μL of solution.

[0105] According to another embodiment, the osmolality of the cellular elements in the solution is between 220 and 360 $\text{mOsmol}\cdot\text{kg}^{-1}$, preferentially between 220 and 360 $\text{mOsmol}\cdot\text{kg}^{-1}$. In this way, the cellular elements and the solution are close to osmotic equilibrium, which means that the ion concentration gradients in the solution are balanced and do not cause osmotic shock.

[0106] Preferably, the composition according to the invention further comprises at least one biocompatible osmolality-adjusting agent. The inclusion of at least one osmolality-adjusting agent allows the osmolality of the solution to be adjusted so that the solution is compatible with the survival of the cellular elements.

[0107] Preferentially, at least one osmolality-adjusting agent of the solution is selected from mannitol, sorbitol, sodium chloride, and mixtures thereof, or modified forms of these molecules.

[0108] According to one embodiment, the composition according to the invention comprises a solution comprising at least one type of ion selected from sodium, calcium, chlorine, magnesium, and potassium ions and mixtures thereof.

[0109] Preferentially, the composition according to the invention comprises a solution comprising sodium, calcium, chlorine, and potassium ions.

5 [0110] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution is an aqueous solution.

10 [0111] According to one embodiment, the composition comprises a solution and cellular elements suspended in said solution, wherein the solution has a pH of between 7.0 and 7.6.

[0112] Advantageously, the pH of the solution is particularly well-suited to the survival of the cellular elements.

15 [0113] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises a biocompatible pH buffer, preferentially selected from phosphate-buffered saline (PBS), citric acid or HEPES, and preferably the PBS buffer referenced under number 4004200 in the European Pharmacopoeia.

20

[0114] According to one variant, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution further comprises at least one active ingredient and/or one pharmaceutically acceptable excipient.

25

[0115] According to one particular embodiment of the invention, the aqueous solution is the culture medium for the cellular elements.

30 [0116] According to another embodiment, the composition according to the invention comprises a solution comprising at least one element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

[0117] Advantageously, the diversity of ions contained in the solution makes it possible to provide a balanced environment that is conducive to the proper functioning of the cellular processes in the cellular elements within the composition according to the invention.

[0118] Preferentially, the composition according to the invention comprises:

- between 0.01 and 0.9% by mass of NaCl with respect to the total mass of the solution; and/or
- 10 - between 0.01 and 0.04% by mass of CaCl_2 with respect to the total mass of the solution; and/or
- between 0.01 and 0.05% by mass of KCl with respect to the total mass of the solution.

15 [0119] Advantageously, the solution and the cellular elements suspended in said solution are close to osmotic equilibrium, ensuring a stable environment for the cellular elements without any risk of osmotic shock.

[0120] Preferentially, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution is isotonic with respect to the cellular elements.

According to one embodiment, the composition according to the invention has a density of between 800 and 2000 kg/m^3 , preferentially of between 997 and 1600 kg/m^3 , more preferentially of between 1025 and 1100 kg/m^3 , in particular of between 1047 and 1090 kg/m^3 , and most preferentially of between 1050 and 1090 kg/m^3

[0121] Advantageously, the density of the composition according to the invention contributes to reducing the maximum sedimentation rate of the cellular elements with respect to the solution.

[0122] According to one variant, the composition according to the invention, comprising a solution and cellular elements in suspension, has a solution density that lies between the density of the cellular element having the lowest density and the density of the cellular element having the highest density.

5

[0123] According to another variant, the composition according to the invention, comprising a solution and cellular elements in suspension, has a solution density lower than the density of the cellular element having the lowest density.

10 [0124] Finally, according to another variant, the composition according to the invention, comprising a solution and cellular elements suspended in said solution, has a solution density greater than the density of the cellular element having the highest density.

15 [0125] Advantageously, in all embodiments according to the invention, the maximum sedimentation rate of the cellular elements with respect to the solution is less than $20 \text{ mm} \cdot \text{h}^{-1}$.

20 [0126] According to another variant, the composition according to the invention has a density equal to the average of the densities of all the cellular elements present in the composition, with a permissible variation of 20 kg/m^3 , preferentially of 10 kg/m^3 . In this way, the density of the composition is centered on the average density of all the cellular elements.

25 [0127] According to one embodiment, the composition according to the invention comprises at least one biocompatible density agent having a molar mass greater than 400 g/mol .

30 [0128] According to a preferred embodiment, the composition according to the invention comprises at least one biocompatible density agent selected from dextran, hypromellose, iodixanol, polyethylene glycol (PEG), chitosan,

pullulan, or molecules of the glycosaminoglycan family, or modified forms of these molecules, and mixtures thereof.

5 [0129] The density agents suitable for the composition according to the invention are selected on the basis of several parameters, namely:

- their biocompatibility, meaning that they must not impair the survival of the cellular elements
- their high solubility in the solution;
- their low effect on the viscosity of the solution.

10

[0130] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises dextran as a biocompatible density agent. When the solution comprises dextran, the dextran preferentially has a molecular weight
15 of between 20 and 80 kDa, particularly of 30, 35, or 40 kDa.

[0131] Carboxymethyl cellulose, hyaluronic acid, or modified forms of these molecules are present in the composition according to the invention and act as viscosity agents, that is, molecules intended to adjust the viscosity without
20 impairing the biocompatibility of the composition according to the invention.

[0132] These molecules have been selected on the basis of several parameters, namely:

- their biocompatibility, meaning that they must not impair the survival of the
25 cellular elements;
- their stability at 37°C, the injection temperature in humans.

[0133] Furthermore, carboxymethyl cellulose, hyaluronic acid, or modified forms of these molecules are particularly suitable when the cells that constitute the
30 cellular elements are neural cells, neuronal cells, glial cells, or mixtures thereof.

[0134] When the solution comprises sodium hyaluronate, it preferentially has a molecular weight of less than 1 MDa, more preferentially of between 80 and 1 MDa, in particular of between 80 and 800 kDa, and even more preferentially of between 80 and 600 kDa.

5

[0135] When the solution comprises carboxymethyl cellulose, the carboxymethyl cellulose preferentially has a molecular weight of between 40 kDa and 1 MDa, and even more preferentially of between 50 and 400 kDa.

10 [0136] Advantageously, all the components of the solution are biocompatible, thereby ensuring that the composition according to the invention will be well tolerated by the body, particularly upon injection.

[0137] According to another embodiment, the composition according to the invention comprises:

15

- between 0.01 and 30% by mass of at least one biocompatible density agent, preferentially dextran, with respect to the total mass of the solution

- between 0.01 and 7% by mass of carboxymethyl cellulose and/or hyaluronic acid and/or modified forms of these molecules, with respect to the total mass

20

- of the solution.

[0138] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises:

25

- sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa and/or carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa; and/or

- dextran having a molecular weight of between 40 and 80 kDa.

30

[0139] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises:

- between 10 and 25% by mass of dextran having a molecular weight of 20 and 80 kDa, with respect to the total mass of the solution;
- between 0.1 and 7% by mass of carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa, with respect to the total mass of the solution, and/or of between 0.1 and 7% by mass of sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa, with respect to the total mass of the solution

[0140] According to a preferred embodiment, the composition according to the invention has a static viscosity, measured at 25°C, of between 0.01 and 1.5 Pa·s, preferentially of between 0.01 and 1 Pa·s, more particularly of between 400 and 1200 mPa·s, more preferentially of between 400 and 1000 mPa·s, and even more preferentially of between 500 and 1100 mPa·s, in particular of between 500 and 900 mPa·s.

[0141] The viscosity of the composition makes it possible to prevent sedimentation of the cellular elements suspended in the solution, while allowing the composition according to the invention to be used, in particular by injecting said composition. Consequently, the composition according to the invention has a viscosity high enough to keep the cellular elements in suspension, while also having a viscosity low enough to minimize frictional forces when the composition is injected, particularly using an injection means such as a syringe or a cannula.

[0142] According to one particular embodiment of the invention, the biocompatible composition has:

- a density of between 997 and 1600 kg/m³;
- a static viscosity, measured using a rheometer at 25°C, of between 0.01 and 1.5 Pa·s.

[0143] Advantageously, all the components of the solution are biocompatible, thereby ensuring that the composition according to the invention will be well tolerated by the body, particularly upon injection.

[0144] According to another embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises:

- 5 - between 10 and 25% by mass of dextran having a molecular weight of 20 kDa and 80 kDa, with respect to the total mass of the solution;
- between 0.01 and 7% by mass of CMC having a molecular weight of between 40 kDa and 1 MDa, with respect to the total mass of the solution, and/or of
- 10 between 0.01 and 7% by mass of sodium hyaluronate having molecular weights of between 80 kDa and 1 MDa, with respect to the total mass of the solution;
- between 0.01 and 0.9% by mass of NaCl with respect to the total mass of the solution; and/or
- between 0.01 and 0.04% by mass of CaCl_2 with respect to the total mass of the solution; and/or
- 15 - between 0.01 and 0.05% by mass of KCl with respect to the total mass of the solution.

[0145] Preferentially, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the maximum
20 sedimentation rate of the cellular elements with respect to the solution is less than $10 \text{ mm} \cdot \text{h}^{-1}$, preferentially less than $5 \text{ mm} \cdot \text{h}^{-1}$, in particular less than $1 \text{ mm} \cdot \text{h}^{-1}$.

[0146] According to one variant, the composition according to the invention comprises a solution and cellular elements suspended in said solution,
25 wherein the maximum relative rate of the cellular elements with respect to the solution is between 0.01 and $20 \text{ mm} \cdot \text{h}^{-1}$.

[0147] Thus, the lower the maximum relative rate of the cellular elements, the better the homogeneity of their distribution in suspension in the solution is
30 maintained over time.

[0148] According to one particular embodiment, the composition according to the invention, which has a maximum sedimentation rate of the cellular elements with respect to the solution of less than $1 \text{ mm} \cdot \text{h}^{-1}$, may be stored for up to 6 hours before use. Thus, in this embodiment, the composition
5 according to the invention maintains the uniform distribution of the heterogeneous cellular elements for 6 hours.

[0149] Advantageously, the composition according to the invention may be stored for a long period of time without affecting the uniform distribution of
10 the heterogeneous cellular elements, thereby ensuring precise dosing, particularly during injection.

[0150] The composition according to the invention may be obtained by any suitable method known to those skilled in the art. However, according to one
15 particularly preferred embodiment, the composition according to the invention is a biocompatible composition obtained according to a method which comprises carrying out the following steps:

(a) Preparing or recovering a composition comprising a set of cellular elements, at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a
20 microtissue having a largest dimension of at least $50 \text{ } \mu\text{m}$;

(b) Preparing a solution comprising:

- hyaluronic acid and/or carboxymethyl cellulose (CMC), and
- at least one biocompatible density agent,
- 25 - and/or at least one modified form of one or more of these molecules;

(c) Sterilizing the solution resulting from step (b) so as to obtain a sterilized filtrate;

(d) Mixing the cellular elements resulting from step (a) with the sterilized filtrate resulting from step (c);

(e) Optionally recovering the biocompatible composition resulting from step
30 (d), comprising a solution and cellular elements suspended in said solution, at least two cellular elements of which have a different density and/or size and/or cell count, said composition comprising the following molecules:

- Hyaluronic acid and/or carboxymethyl cellulose (CMC);
- At least one density agent;
- and/or at least one modified form of one or more of these molecules.

5 [0151] According to one embodiment, the set of cellular elements of step (a) comprises neural cells, neuronal cells, and/or glial cells, preferentially dopaminergic neurons.

[0152] Preferentially, when step (a) relates to the preparation of a
10 composition, it comprises carrying out the following steps:

- 1) Culturing at least one cellular element;
- 2) Rinsing the culture of cellular elements resulting from step 1);
- 3) Centrifuging the culture of cellular elements resulting from step 2);
- 4) Recovering a composition comprising cellular elements resulting from the
15 centrifugation of step 3), at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 μm .

[0153] According to one embodiment, the composition of step (a) comprises
20 a solution in which said cellular elements are suspended.

[0154] Preferably, the solution of step (b) comprises:

- between 0.1 and 7%, preferentially of between 4 and 7% by mass, of sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa, with
25 respect to the total mass of the solution, and/or of between 0.1 and 7% by mass of carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa, with respect to the total mass of the solution; and
- between 10 and 25% by mass of at least one biocompatible density agent with respect to the total mass of the solution.

30

[0155] Preferentially, step (b) further comprises adding at least one element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

5 Method for preparing a biocompatible composition according to the invention

[0156] According to another aspect, the invention also relates to a method for preparing a composition according to the invention comprising carrying out the following steps:

- 10 (a) Preparing or recovering a composition comprising a set of cellular elements, at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 µm;
- (b) Preparing a solution comprising:
- 15 - hyaluronic acid and/or carboxymethyl cellulose (CMC), and
- at least one biocompatible density agent,
- and/or at least one modified form of one or more of these molecules;
- (c) Sterilizing the solution resulting from step (b) so as to obtain a sterilized filtrate;
- (d) Mixing the cellular elements resulting from step (a) with the sterilized
- 20 filtrate resulting from step (c);
- (e) Optionally, recovering the biocompatible composition resulting from step (d), comprising a solution and cellular elements suspended in said solution, at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest
- 25 dimension of at least 50 µm, said composition comprising the following molecules:
- Hyaluronic acid and/or carboxymethyl cellulose (CMC);
 - At least one density agent;
 - and/or at least one modified form of one or more of these molecules.

- 30 [0157] Advantageously, the method according to the invention makes it possible to obtain an injectable biocompatible composition that exhibits homogeneous distribution of cellular elements and biological stability for a period of up to 6 hours.

[0158] According to one embodiment, the composition of step (a) comprises a solution in which said cellular elements are suspended.

- 5 [0159] According to one preferred embodiment, the composition of step (a) has a maximum sedimentation rate of the cellular elements with respect to the solution greater than $20 \text{ mm} \cdot \text{h}^{-1}$.

[0160] Preferably, the composition of step (a) comprises cellular elements
10 comprising:

- neuronal cells, preferentially dopaminergic neurons; and/or - glial cells, preferentially astrocytic cells and/or oligodendrocytic cells; and/or
- neural cells such as progenitor cells.

- 15 [0161] According to one embodiment, the composition of step (a) comprises at least two distinct cell types.

[0162] Preferentially, when step (a) relates to the preparation of a composition, it comprises carrying out the following steps:

- 20 1) Culturing at least one cellular element;
2) Rinsing the culture of cellular elements resulting from step 1);
3) Centrifuging the culture of cellular elements resulting from step 2);
4) Recovering a composition comprising cellular elements resulting from the centrifugation of step 3), at least two cellular elements of which have a
25 different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least $50 \text{ } \mu\text{m}$.

[0163] According to one embodiment, the cell culture described in step 1) is carried out in a bioreactor.

30

[0164] According to one particular embodiment of the invention, at least one cellular element of step 1) comprises at least one encapsulated cell, preferentially encapsulated in alginate.

- 5 [0165] Preferably, at least one cellular element from step 1) is contained in a three-dimensional microcompartment comprising an outer hydrogel layer.

[0166] When step 1) comprises culturing at least one encapsulated cellular element, step (a) may also comprise a step 2') aimed at removing the capsule
10 in order to recover the cellular element prior to centrifugation step 3).

[0167] Step 2') consists in removing the capsule, preferentially removing the outer hydrogel layer. Preferentially, step 2') may be carried out, in particular, by hydrolysis, dissolution, perforation, and/or rupture using any
15 biocompatible means, i.e., means that are non-toxic to the cells. For example, the removal may be carried out using a phosphate-saline buffer, a divalent ion chelator, an enzyme such as alginate lyase if the hydrogel contains alginate, and/or laser microdissection.

- 20 [0168] According to one embodiment, step (b) comprises preparing a solution in which at least one biocompatible density agent has a molar mass greater than 400 g/mol.

[0169] Preferentially, at least one biocompatible density agent of step (b) is
25 selected from dextran, hypromellose, iodixanol, polyethylene glycol (PEG), chitosan, pullulan, or molecules of the glycosaminoglycan family, or modified forms of these molecules, and mixtures thereof.

[0170] In a particularly preferred embodiment, the solution resulting from step
30 (b) comprises dextran as the density agent.

[0171] According to a preferred embodiment, the solution resulting from step (b) comprises:

- between 0.1 and 7%, preferentially of between 4 and 7% by mass, of sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa, with respect to the total mass of the solution, and/or of between 0.1 and 7% by mass of carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa, with respect to the total mass of the solution; and
- between 10 and 25% by mass of at least one biocompatible density agent with respect to the total mass of the solution.

10

[0172] According to another particular embodiment, the mixture of step (b) comprises:

- between 10 and 20% by mass of dextran, with respect to the volume of the solution, preferentially having a molecular weight of 20 kDa and 80 kDa;
- between 3 and 7% by mass of CMC preferentially having a molecular weight of between 40 kDa and 1 MDa, with respect to the volume of the solution, and/or between 0.3 and 7% by mass of sodium hyaluronate having a molecular weight of between 80 and 800 kDa.

- 20 [0173] Preferably, step (b) further comprises adding at least one element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

25 [0174] According to one embodiment, the solution resulting from step (b) comprises:

- between 0.01 and 0.9% by mass of NaCl with respect to the total mass of the solution; and/or
- between 0.01 and 0.04% by mass of KCl with respect to the total mass of the solution; and/or
- between 0.01 and 0.05% by mass of CaCl₂ with respect to the total mass of the solution.

[0175] Preferentially, the sterilization of step (c) is carried out by filtration through a mesh having a pore size of between 0.01 and 0.22 μm .

5 [0176] According to one embodiment, the method according to the invention comprises a step (c'), performed after step (c), of adjusting the pH of the sterilized filtrate resulting from step (c), so that the filtrate resulting from step (c') has a pH of between 6.8 and 8, preferentially of between 7 and 7.6.

10 [0177] According to another embodiment, the method according to the invention comprises a step (c'), performed prior to step (c), of adjusting the pH of the solution resulting from step (b), such that the solution resulting from step (c') has a pH of between 6.8 and 8, preferentially of between 7 and 7.6.

15 [0178] According to one preferred embodiment, step (c') consists in adding a biocompatible pH buffer, preferentially selected from phosphate buffer, citric acid, or HEPES, and more preferably the phosphate-saline buffer referenced under number 4004200 in the European Pharmacopoeia, to the sterilized filtrate from step (b3) so that the sterilized filtrate has a pH of between 6.8 and 8, preferentially of between 7 and 7.6.

20

[0179] According to one embodiment, step (d) is carried out with stirring, preferentially using a paddle mixer or a magnetic stir bar.

25 [0180] According to one embodiment, step (d), which involves mixing the cellular elements of the composition of step (a) with the sterilized filtrate from step (c) or, optionally, step (c'), comprises a step of extracting the cellular elements from the composition of step (a).

30 [0181] According to one embodiment, the mixture resulting from step (d) has:
- a density of between 800 and 2000 kg/m^3 , preferentially of between 1047 and 1090 kg/m^3 , and more preferentially of between 1050 and 1090 kg/m^3 ; and

- a static viscosity, measured using a rheometer at 25°C, of between 0.01 and 1.5 Pa·s, preferentially of between 600 and 1200 mPa·s, preferentially of between 500 and 900 mPa.

- 5 [0182] Advantageously, a person skilled in the art may be able to tailor the density and viscosity by varying the concentrations of at least one density agent and/or dextran.

[0183] Thus, according to one embodiment, the method for preparing a
10 composition according to the invention comprises carrying out the following steps:

(a) Preparing a composition comprising a set of cellular elements in a solution, at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 µm, comprising the following steps:

- 15 1) Culturing at least one cellular element;
2) Rinsing the culture of cellular elements resulting from step 1);
3) Centrifuging the culture of cellular elements resulting from step 2);
4) Recovering a composition comprising cellular elements resulting from the centrifugation of step 3), at least two cellular elements of which have a
20 different density and/or size and/or cell count;
- (b) Preparing a solution comprising:
- hyaluronic acid and/or carboxymethyl cellulose (CMC), and
 - at least one biocompatible density agent,
 - and/or at least one modified form of one or more of these molecules;
- 25 (c) Sterilizing the solution resulting from step (b) so as to obtain a sterilized filtrate;
(c') Adjusting the pH of the sterilized filtrate resulting from step (c) so that the filtrate resulting from step (c') has a pH of between 6.8 and 8;
- (d) Mixing, with stirring, the cellular elements resulting from step (a) with the sterilized filtrate resulting from step (c);
- 30 (e) Optionally recovering the biocompatible composition resulting from step (d), comprising a solution and cellular elements suspended in said solution,

at least two cellular elements of which have a different density and/or size and/or cell count, said composition comprising the following molecules:

- Hyaluronic acid and/or carboxymethyl cellulose (CMC);
- At least one density agent;

5 - and/or at least one modified form of one or more of these molecules.

[0184] Preferably, step (b) is carried out such that the solution resulting from step (b) has:

- a density of between 0.997 and 1.6 g/ml; and
- 10 - a static viscosity, measured using a rheometer at 25°C, of between 0.01 and 1.5 Pa·s, preferentially of between 0.01 and 1.2 Pa·s.

[0185] According to one embodiment, the method according to the invention comprises a step of cryopreserving the composition resulting from step (a).

15 When the method according to the invention comprises a step of cryopreserving the composition resulting from step (a), said method further comprises a step of thawing the cryopreserved composition.

[0186] According to one embodiment, the method for preparing a composition
20 according to the invention comprises carrying out the following steps:

(a) Preparing a composition comprising a set of cellular elements in a solution, at least two cellular elements of which have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 µm, comprising the following steps:

- 25 1) Culturing at least one cellular element;
- 2) Rinsing the culture of cellular elements resulting from step 1);
- 3) Centrifuging the culture of cellular elements resulting from step 2);
- 4) Recovering a composition comprising cellular elements resulting from the centrifugation of step 3), at least two cellular elements of which have a
30 different density and/or size and/or cell count;

(b) Preparing a solution comprising:

- hyaluronic acid and/or carboxymethyl cellulose (CMC), and

- at least one biocompatible density agent,
- and/or at least one modified form of one or more of these molecules;

(c') Adjusting the pH of the solution resulting from step (b) so that the solution resulting from step (c') has a pH of between 6.8 and 8;

5 (c) Sterilizing the solution resulting from step (c') so as to obtain a sterilized filtrate;

(d) Mixing, with stirring, the cellular elements resulting from step (a) with the sterilized filtrate resulting from step (c);

(e) Optionally recovering the biocompatible composition resulting from step (d), comprising a solution and cellular elements suspended in said solution,

10 at least two cellular elements of which have a different density and/or size and/or cell count, said composition comprising the following molecules:

- Hyaluronic acid and/or carboxymethyl cellulose (CMC);
- At least one density agent;
- and/or at least one modified form of one or more of these molecules.

15

Composition according to the invention for use:

[0187] According to another aspect, the invention relates to the composition according to the invention as described in any one of the embodiments
20 previously described, for use as a drug.

[0188] Advantageously, the biocompatible composition according to the invention exhibits rheological characteristics and a maximum sedimentation rate of the cellular elements that are particularly well-suited for use in cell therapy.

25

[0189] Preferably, the composition may be used in the treatment of diseases by cell therapy.

[0190] According to one preferred embodiment, the composition may be used
30 in the treatment of Parkinson's disease.

[0191] According to one embodiment, the composition according to the invention comprises cellular elements, preferentially in the form of microtissue(s), comprising at least neural cells, neuronal cells, glial cells, or mixtures thereof, for use in the treatment of Parkinson's disease.

5

[0192] According to one preferred embodiment, the composition according to the invention comprises cellular elements comprising at least one dopaminergic neuron for use in the treatment of Parkinson's disease.

10 [0193] Advantageously, the biocompatible composition according to the invention exhibits rheological characteristics and a maximum sedimentation rate of the cellular elements, preferentially dopaminergic neurons, that are particularly well-suited for use in cell therapy.

15 [0194] According to another embodiment, the composition according to the invention, comprising a solution and cellular elements suspended in said solution, wherein at least 70% of the cellular elements are in the form of microtissue comprising dopaminergic neurons, may be used in the treatment of Parkinson's disease.

20

[0195] According to one variant of the invention, the composition according to the invention comprising a solution and cellular elements suspended in said solution, wherein:

- at least 50% of the cellular elements are in the form of microtissue;

25 - less than 20% of the cellular elements are in the form of isolated cells; and

- at least 30% of the cellular elements are dopaminergic neurons

may be used in the treatment of Parkinson's disease.

30 [0196] Advantageously, when the composition according to the invention comprises microtissues, said microtissues recreate the tissue architecture and the cellular interactions necessary to promote tissue regeneration. Cell clusters, for their part, provide a microenvironment conducive to cell survival and

differentiation. Furthermore, the injection of a composition comprising heterogeneous cellular elements provides cellular diversity and potentially improved cellular plasticity.

- 5 [0197] In the context of the invention, the composition according to the invention is particularly well-suited for use as a biocompatible injectable composition for treating Parkinson's disease.

- 10 [0198] According to one embodiment, the composition according to the invention may be intended for multiple uses. In other words, the composition may be used to perform multiple injections while controlling the amount of cells injected and maintaining the viability of the cellular elements, even if the injections are spaced apart by a period of several hours.

- 15 [0199] Advantageously, the maximum sedimentation rate of the cellular elements makes it possible to inject, for a constant volume, an amount of cellular elements having a low standard deviation, preferentially a standard deviation of less than 20%.

- 20 [0200] According to one embodiment, the composition according to the invention may be administered by injection using an injection means at the injection site.

[0201] Preferentially, said injection means is a cannula.

- 25 [0202] Advantageously, controlling the rheological characteristics of the composition according to the invention makes it possible to optimize the administration of the cellular elements and, consequently, of the therapeutic cells, in a precise and effective manner. Controlling these parameters makes it possible to tailor the injection flow rate and the injection volume, thereby ensuring
30 homogeneous distribution of the cellular elements within the target area.

[0203] According to one embodiment, the composition according to the invention may be administered by injection using a cannula at the injection site, preferentially into the putamen of a human or animal.

5 Kit 1:

[0204] According to another aspect, the invention relates to a kit comprising:

- a biocompatible composition according to any one of the embodiments previously described; and

10 - an injection means, preferentially a cannula.

[0205] Advantageously, the kit according to the invention provides the practitioner with all the elements necessary for optimizing the effectiveness of the injection of the cellular elements.

15

[0206] According to one embodiment, the kit according to the invention comprises:

- a cannula

- a biocompatible composition according to any one of the embodiments previously described, wherein the at least one cellular element has a largest dimension of less than 0.8 times the smallest internal diameter of the cannula, preferentially of less than 0.75 times the smallest internal diameter of the cannula, in particular of between 0.2 and 0.7 times the smallest internal diameter of the cannula.

25

[0207] According to one particular embodiment of the invention, the kit according to the invention comprises a biocompatible composition according to any one of the embodiments previously described in solid form.

30 [0208] Preferentially, the kit according to the invention further comprises water.

[0209] According to one embodiment, the kit according to the invention further comprises an element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

5

Kit 2:

[0210] Finally, according to a final aspect, the invention relates to a kit comprising:

* a preparation (1), in solid form, comprising

- 10 - hyaluronic acid and/or carboxymethyl cellulose (CMC), and
 - at least one biocompatible density agent,
 - and/or at least one modified form of one or more of these molecules.

15 * a preparation (2) comprising cellular elements, said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and mixtures thereof, wherein at least two of said cellular elements have a different density and/or size and/or cell count; preferentially, at least one cellular element is a microtissue having a largest dimension of at least 50 µm.

[0211] Preferentially, the kit according to the invention further comprises water.

20

[0212] According to one embodiment, the preparation (1), in solid form, comprises at least one biocompatible density agent selected from dextran, hypromellose, iodixanol, polyethylene glycol (PEG), chitosan, pullulan, or molecules of the glycosaminoglycan family, or modified forms of these
25 molecules, and mixtures thereof.

[0213] Preferentially, the preparation (1) comprises dextran as a biocompatible density agent.

30 [0214] According to one embodiment, the kit according to the invention further comprises an element selected from sodium chloride (NaCl), calcium chloride

(CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

EXAMPLES:

5

Example 1:

[0215] The objective of this example is to evaluate the sedimentation rate of the cellular elements in different compositions, namely a composition comprising cellular elements and a Hank's balanced saline solution (HBSS) (A) (comparative), and the composition according to the invention comprising a solution (B) developed to maintain the homogeneous distribution of the cellular elements and prevent clogging of the injection system.

10
15 [0216] After the cellular elements were obtained, they were cryopreserved in vials and stored in liquid nitrogen. The vials containing the elements were then thawed, with an estimated 10 million cells.

[0217] In the context of this example, the cellular elements consist of a mixture of microtissues having a largest dimension of at least 50 µm, isolated cells, and clusters of neuronal cells.

[0218] The results of the cellular element characterization made it possible to measure the density and the largest dimension of the cellular elements, namely:
25 Density of the cellular elements: 1056 kg/m³;
Largest dimension of the cellular elements: 200 µm, i.e. 0.0002 m.

[0219] Solution (B), described in Table 1 below, was prepared under sterile conditions and characterized in Table 2.

30

[Table 1]

Reagent	Reference	Test concentration
HA100	PHI4468P02E (I.V.: 0.22%)	48 mg/mL
Dextran 40	CAS 9004-54-0	130 mg/mL
KCl	CAS 7447-40-7	0.30 mg/mL
CaCl ₂ ·2H ₂ O	CAS 10035-04-8	0.15 mg/mL
C ₆ H ₈ O ₇	CAS 77-92-9	0.25 mg/mL
Na ₂ HPO ₄	CAS 7558-79-4	2.50 mg/mL

[Table 2]

Physicochemical parameter of solution (B)	Value
Static viscosity	0.454 Pa·s
Density	1049 kg/m ³
pH	7.37
Osmolarity	216 mOsmol

- 5 [0220] Solution (A) was also prepared under sterile conditions and characterized in Table 3.

[Table 3]

Physicochemical parameter of solution (B)	Value
Static viscosity	0.0012 Pa·s
Density	1002 kg/m ³

10

[0221] Preparation of the compositions:

Phase 1: Preparation of the cellular elements

- 15 [0222] The vials were removed from the liquid nitrogen and placed in a CryoPod at -180°C. The vials comprising the cellular elements were placed in a thermostatic chamber set to 37°C and removed when only a small piece of ice remained (≈2 min).
- 20 [0223] After gently resuspending the cells, the contents were transferred to a 25-ml tube containing 9 ml of HBSS (calcium, magnesium, glucose) at room temperature.

[0224] The vials were rinsed with 1 ml of HBSS (calcium, magnesium, glucose) in order to recover the maximum amount of cellular elements

5 [0225] The 25-ml tube was centrifuged at $300 \times g$ for 5 minutes, and as much of the supernatant as possible was removed using a P1000 filter followed by a P20 filter.

[0226] The cellular elements were resuspended in 1 mL of HBSS (calcium, magnesium, glucose).

10

Phase 2: Aliquoting the samples

[0227] An undetermined volume and concentration of the cell suspension were aliquoted into two 1.5-ml Eppendorf tubes.

15

[0228] The 1.5-ml tubes were centrifuged at $300 \times g$ for 5 min. and as much of the supernatant as possible was removed using a P1000 filter followed by a P20 filter.

20 [0229] In one sample, the cellular elements were resuspended in 1 mL of HBSS (solution (A)), and in the other, in 1 mL of solution (B).

Phase 3: Evaluation of the sedimentation rate

25 [0230] The tubes were installed on the assembled apparatus so that photos could be taken.

[0231] Photos were taken every minute for 12 hours; see Figure 1.

30 [0232] Using ImageJ software, the trajectories of the cellular elements were manually tracked from a time-lapse video recorded with the camera in order to evaluate the sedimentation rate.

[0233] An average of five sedimentation rates of the cellular elements in solution (B), measured over 10/12 trajectories, was calculated to estimate the maximum sedimentation rate. In solution (A), only three rates of cellular elements, measured
 5 over four trajectories, were calculated to determine the value, due to the rapid sedimentation and the difficulty of tracking exactly the same cellular element.

[0234] The calculated maximum sedimentation rate (average of the five maximum rates for solution (B) and average of the three maximum rates for
 10 solution (A) of microtissues) is:

* approximately 363 mm/h in comparative solution (A);

* approximately 1.66 mm/h in solution (B) according to the invention, wherein the difference between the density of the solution and the average density of the cellular elements of the composition resulting from step (b5) satisfies the
 15 inequality [Math 1].

[0235] Table 4 below satisfies the inequality [Math 1] for solution (A) and solution (B) with $V_c = 5.56 \times 10^{-6} \text{m} \cdot \text{s}^{-1}$.

20 [Table 4]

	$ \Delta\rho = \rho_{MT} - \rho_s$	$F \cdot v_c / D^2$	$ \Delta\rho < F \cdot v_c / D^2$
Comparative solution (A)	54	0.03	NO
Inventive solution (B)	7	11.6	YES

Conclusions:

25 [0236] The composition according to the invention comprising the cellular elements and solution (B) makes it possible to maintain said cellular elements in suspension in said solution (B) for several hours. Within the composition according to the invention, some of the cellular elements remain suspended after 12 hours of sedimentation.

30

[0237] In contrast, the comparative composition comprises the cellular elements and solution (A) does not make it possible to maintain the cellular elements suspended in said solution (A) over an extended period. The cellular elements sediment very rapidly in solutions having low viscosity and no density matching, such as HBSS (calcium, magnesium, glucose), which has a static viscosity of approximately 1 mPa·s and a density of approximately 1.0038 g/cm³

Example 2:

[0238] The objective of this example is to evaluate the viability of the cellular elements after 12 hours of sedimentation within the composition according to the invention.

[0239] In order to carry out this example, the composition according to the invention comprises the following elements:

[Table 5]

Reagent	Reference	Test concentration
HA100	PHI4468P02E (I.V.: 0.22%)	5.4%
Dextran 40	CAS 9004-54-0	12%
KCl	CAS 7447-40-7	0.03%
CaCl ₂ ·2H ₂ O	CAS 10035-04-8	0.015%
C ₆ H ₈ O ₇	CAS 77-92-9	0.025%
Na ₂ HPO ₄	CAS 7558-79-4	0.25%

[0240] The composition was also prepared under sterile conditions and characterized in Table 6.

[Table 6]

Physicochemical parameter	Value
Static viscosity	0.62 Pa·s
Density	1,073.5 kg/m ³
pH	7.34
Osmolarity	211 mOsmol

[0241] The results of the cellular element characterization made it possible to measure the density and the largest dimension of the cellular elements, namely:

Density of the cellular elements: 1065 kg/m^3 ;

- 5 Largest dimension of the cellular elements: $200 \text{ }\mu\text{m}$, i.e. 0.0002 m ;

In the context of this example, the sedimentation time is 12 hours, which corresponds to the maximum stability limit when the composition according to the invention is used in cell therapy.

10 Experimental protocol

Conditions

- 15 [0242] The production batches are identified by the prefix "PAR," followed by an incremental indicator ranging from 19 to 22. They correspond to successive production batches of cellular elements.

1) For each batch, two types of samples are prepared:

- 20 Control: A suspension of cellular elements resuspended in a reference cell culture medium, at a ratio of 20% by volume of cellular elements and 80% of reference culture medium.

Composition according to the invention: A suspension of cellular elements resuspended in a solution, at a ratio of 20% by volume of cellular elements to 80% by volume of solution.

- 25 2) Once the samples have been prepared, they are stored in sealed tubes at room temperature for 12 hours.

3) The cellular elements are then rinsed in an excess of reference culture medium.

4) The cellular elements are then returned to culture for 24 hours in a reference culture medium.

- 30 5) Viability is measured. The result is expressed by normalizing the viability value under the immersion condition in the solution by the viability obtained in the control condition.

Results

[0243] The results of this example are shown in Figure 2. This experiment shows an average decrease in viability of approximately 25% after immersion for 12 hours in the solution under consideration, with respect to a 12-hour control in a reference culture medium. In the context of this example, this decrease in viability is acceptable given the acceptance criterion of 70% relative viability.

Example 3: Evaluation of the impact of the procedure for injecting the composition according to the invention on the viability of the cellular elements.

[0244] The objective of this example is to evaluate the impact of the procedure for injecting the composition according to the invention on the viability of the cellular elements.

[0245] In the context of the invention, it is important to verify that the viscosity of the solution, which is necessary for maintaining the homogeneous distribution of the cellular elements, does not induce excessively high shear stresses during injection, which could compromise the viability of the cells.

[0246] The experiment consists in comparing the viability of a known volume of the composition according to the invention that has undergone an injection procedure under the conditions of the invention with the viability of a known volume of the composition according to the invention that has not undergone an injection procedure.

[0247] Since the shear stresses experienced during injection may have a delayed effect on cell viability, the viability of the cellular elements that have undergone an injection procedure is measured immediately after injection and then after 24 hours of returning to culture in a reference culture medium.

[0248] The composition according to the invention used in this example has the same composition (Table 5) and the same rheological characteristics (Table 6) as the composition according to the invention described in Example 2.

5 [0249] The injection protocol comprises the following steps:

a) Preparing the composition according to the invention using the method according to the invention;

b) Filling a syringe with the composition according to the invention;

10 c) Injecting the composition according to the invention using the syringe from step b) into a cannula at an injection flow rate of 50 $\mu\text{L}/\text{min}$;

d) Injecting the composition according to the invention, using the cannula, onto a matrix at a flow rate of between 18 μL and 2 μL per minute;

e) Rinsing the composition according to the invention and resuspending the cellular elements in a culture medium;

15 f) Placing the culture medium containing the cellular elements in an incubator for 24 hours.

[0250] The viability of the cellular elements is measured at the end of step e) and at the end of step f). The experiment was conducted three times.

20

Results:

[0251] The viability measurements shown in Figure 3 do not demonstrate any significant impact of the injection procedure on viability under the conditions of the example. The slight decrease observed immediately after injection may reflect a transient decline in the metabolic activity of the cells; however, this decrease is not observed after 24 hours of culture.

25

[0252] Consequently, the composition according to the invention may be used as an injectable composition.

30

Example 4: Characterization of the viscoelastic properties of a composition according to the invention via an oscillatory sweep rheological measurement

[0253] To carry out this example, the composition according to the invention
5 comprises a solution characterized in Table 7, as well as cellular elements.

[Table 7]

Reagent	Wt. %	Concentration
HA500	1.00	10.7 mg/mL
Dextran D40	15.00	160.50 mg/mL
KCl	0.003	1.61 mg/mL
Sodium chloride	0.15	0.32 mg/mL
Calcium chloride dihydrate	0.013	0.14 mg/mL
Disodium phosphate	0.30	3.21 mg/mL
Citric acid	0.025	0.27 mg/mL

10 [0254] The cellular elements of the composition used in the context of this example are a mixture of microtissues having a largest dimension of at least 50 μm , isolated cells, and clusters of neuronal cells.

[0255] Finally, some physicochemical parameters of the composition
15 according to the invention are characterized in Table 8 below.

[Table 8]

Physicochemical parameter of the composition	Value
Static viscosity	900 mPa·s
Density	1071 kg/m ³
pH	7.2

20 [0256] The behavior of the composition according to the invention was verified by conventional rheological measurements using oscillatory sweep analysis with an Anton Paar MCR 302 rheometer. The angular frequency is constant and set at 10 rad/s. The shear deformation varies from 0.001 to 10%, and the temperature is constant and maintained at 20°C.

25

[0257] Two parameters were measured to characterize the viscoelastic properties of the composition: the storage modulus (G') and the loss modulus (G''):

- The storage modulus (G') represents the elastic portion of the deformation. It is related to the ability of the material to store energy during deformation and is proportional to the rigidity of the material. It therefore determines the magnitude of its elastic response and, consequently, relates to the behavior of an elastic solid, that is to say, of a gel.

- The loss modulus (G'') represents the viscous component of the deformation. It is related to the dissipation of energy in the form of heat and is proportional to the damping behavior of the material. It determines the magnitude of its viscous response and, consequently, relates to the behavior of a fluid.

[0258] In the case of a rigid or structured material, the storage modulus (G') is greater than the loss modulus (G''). Conversely, in the case of a liquid material, the loss modulus (G'') is greater than the storage modulus (G').

[0259] The results presented in Figure 4 demonstrated that G'' remained constant across different shear strains at a constant temperature and was always less than G' , indicating that the composition exhibited liquid behavior.

Example 5: Study of the toxicity of the composition according to the invention via intracerebral injection into the striatum of athymic nude rats over a period of 28 days.

[0260] To carry out this example, two compositions were used, namely:

Condition A: A composition comprising the solution used in Example 4 and characterized in Table 8;

Condition B: The composition according to the invention comprising the solution used in Example 4 and characterized in Table 8, and cellular elements, said cellular elements being a mixture of microtissues having a largest dimension of at least 50 μm , isolated cells, and clusters of neuronal cells.

1. Protocol

a. Animal treatment

- 5 [0261] On Day 0, the rats underwent surgery to administer four stereotactic injections during the same procedure.

[0262] 4 μ L of the composition according to the invention was injected per trajectory. The rats were administered a subcutaneous injection of buprenorphine
10 (0.05 mg/kg) a few minutes before anesthesia. The rats were then anesthetized using an induction chamber with 4% isoflurane, and the level of anesthetic was monitored and controlled by regularly checking the plantar reflex.

[0263] Once the animal was anesthetized, analgesia was provided by
15 multiple subcutaneous injections of lidocaine (up to 4 mg/kg) at the incision site. The rats were then injected with the composition according to Condition A (without cellular elements) and according to Condition B (with cellular elements) into the right striatum using a 25- μ L Hamilton syringe with custom-made needles (glass cannula).

20

[0264] The injection was performed using four trajectories with a stereotactic frame. At the end of the surgery, Metacam was administered subcutaneously at a concentration of 1 mg/kg for postoperative pain management.

25 b. Clinical follow-up and observations

[0265] Clinical signs were monitored daily from D0 (before treatment) until sacrifice.

- 30 [0266] The clinical examination involved observing the following parameters: changes in mobility, gait, behavior, and respiration; changes in the condition

of the eyes, skin, coat, and mucous membranes; and the appearance of tumors, diarrhea, and excretion.

c. Body weight measurement

5

[0267] The animals were weighed twice a week from Day 0 until the end of the study. Body weight was also measured before sacrifice. Changes in body weight were assessed relative to the maximum weight recorded for each animal. The results are summarized in Tables 10 and 11.

10

d. Food and water intake

[0268] For each cage, the rats' food intake was measured daily from D0 until the end of the study. The results are summarized in Table 12.

15

e. Blood draws and tests

[0269] Blood samples were collected on D-7, D-2 (pre-dose), D2, D7, and at sacrifice (D7 or D28). At least 1 mL of blood was collected from the caudal artery or via intracardiac sampling on the day of sacrifice. The rats were fasted for at least 16 hours the day before sampling. 200 μ L of blood was placed in an EDTA tube for hematological analysis. The remainder of the sample was allowed to clot and then centrifuged to obtain serum. The serum was divided into two portions for biochemical analysis and CRP analysis, which was quantified by ELISA in accordance with the manufacturer's instructions. The results are summarized in Table 13

20

25

f. Histopathological analysis

[0270] The animals were administered buprenorphine (0.05 mg/kg) subcutaneously after being anesthetized with isoflurane. The animals were then infused with 0.9% NaCl at a rate of 25 mL/min via an intracardiac route.

30

After extraction, the brains were placed in 4% PFA at 4°C for 72 hours and then stored in calcium- and magnesium-free DPBS at 4°C.

5 [0271] The brains were next embedded in OCT and then embedded in paraffin. 5-µm-thick sections were prepared using a microtome, then deparaffinized and stained with HES (hematoxylin, eosin, and safranin for tissue morphology and structure) and MTG (Masson-Goldner trichrome for the visualization of connective tissues) in order to enable a histopathological assessment of the safety of the solution.

10

[0272] The stained sections were imaged using an Axio Scan.Z1 scanner and evaluated by a pathologist in accordance with the guidelines of the European RITA database (Registry of Industrial Toxicology Animal-data) for Levels I and II.

15 2. Results

[0273] The study aims to evaluate the potential toxicity of the composition according to the invention when administered via a stereotactic injection system into the striatum of immunodeficient Rowett nude rats.

20

[0274] Ten immunodeficient male nude rats received bilateral administration of the composition according to the invention using two trajectories (4 µL per trajectory/site) per hemisphere.

25 [0275] The rats were observed daily for mortality, morbidity, and any obvious signs of toxicity. During treatment, one animal from the “D28 sacrifice” group died under anesthesia on the day of surgery. It was concluded that the death was the result of the anesthesia and was not related to the injection of the solution. No animals died during the study, and no animals displayed any signs of toxicity that
30 could be attributed to treatment with the composition according to the invention.

[0276] During the first week, a slight weight loss was observed, likely due to blood draws and surgery. A slight increase in body weight was then observed in the animals after the first week (Tables 9 and 10). Based on an analysis of the average body weight data, treatment with the solution had no effect on the body weight of the animals.

[Table 9]: Average body weights of rats between D-10 and D7 (expressed in grams, average $\pm$ SD, median; N = number of animals, D = day)

		D-10	D0	D1	D4	D7
Condition A	D7 sacrifice group (g)	294.3 $\pm$ 38.3	283 $\pm$ 37	280.1 $\pm$ 34.7	271.3 $\pm$ 35.4	256.2 $\pm$ 36
	Median	292.2	276.0	272.0	265.0	244.6
	N	5	5	5	5	5
	D28 sacrifice group (g)	301.6 $\pm$ 26.1	276.9 $\pm$ 15.7	298.3 $\pm$ 42.8	257.8 $\pm$ 8	285 $\pm$ 39.2
	Median	297.7	272.6	281.7	254.2	272.8
	N	4	4	3	3	3
Condition B	D7 sacrifice group (g)	309.1 $\pm$ 42.4	308.1 $\pm$ 52.4	282.8 $\pm$ 34.9	272.4 $\pm$ 34.2	276.4 $\pm$ 44.4
	Median	294.3	306.4	285.4	271.0	269.8
	N	5	5	4	4	5
	D28 sacrifice group (g)	306 $\pm$ 29.6	332.5 $\pm$ 55.1	286.7 $\pm$ 32.3	288.4 $\pm$ 38.5	267.2 $\pm$ 27.5
	Median	304.5	348.9	294.9	294.8	251.8
	N	5	3	4	3	5

[0277] No significant difference was observed between condition A and condition B.

[Table 10] Average body weights of rats between D-10 and D28 (expressed in grams, average $\pm$ SD, median; N = number of animals, D = day)

		D-10	D0	D1	D2	D3	D5-6	D10-11	D14-16	D17-19	D20-22	D23-25	D26-28
Condition A	D28 sacrifice group (g)	301.6 ± 26.1	276.9 ± 15.7	298.3 ± 42.8	277.3 ± 37.7	257.8 ± 8	285 ± 39.2	267.9 ± 12	304.7 ± 42	318.1 ± 44.8	313.3 ± 50.4	297.9 ± 32.5	337.1 ± 95.5
	Median	297.7	272.6	281.7	261.2	254.2	272.8	262.6	288.5	304.2	299.5	297.3	337.1
	N	4	4	3	3	3	3	3	3	3	3	3	3
Condition B	D28 sacrifice group (g)	300 ± 29.6	332.5 ± 55.1	286.7 ± 32.3	244.2 ± 29.5	288.4 ± 38.5	267.2 ± 27.5	303.6 ± 38.4	314 ± 39.7	323 ± 37.9	329.2 ± 39.2	348.3 ± 41.6	333.6 ± 30.4
	Median	304.5	348.9	294.9	235.0	294.8	251.8	307.3	320.4	332.0	337.1	347.7	328.9
	N	5	5	3	3	4	3	4	5	5	5	4	3

[0278] Average food intake is shown in Table 11. On each day of measurement, food intake was comparable among the animals. The average for the group sacrificed on D7 was 18.2 ± 7.4 g per rat per day, and 24.8 ± 11.0 g per rat per day for the group sacrificed on D28, consistent with the normal intake of an adult rat (between 15 and 20 g per day for an adult rat).

[0279] No significant difference was observed between condition A and condition B.

10

[Table 11]: Food intake (expressed in grams per day per rat; D = day; NA = not applicable)

		Up to D7	Up to D28	During the study
Condition A	D7 sacrifice group (g)	18.2	NA	NA
	Standard deviation	7.4	NA	NA
	D28 sacrifice group	26.6	24.3	24.8
	Standard deviation	18.2	3.3	11.0
Condition B	D7 sacrifice group (g)	19.6	NA	NA
	Standard deviation	4.8	NA	NA
	D28 sacrifice group	20.5	25.6	23.7
	Standard deviation	4.4	3.2	4.4

[0280] Inflammation was monitored by measuring CRP; the results are presented in Table 12. No significant differences were observed among the animals, and no increase in CRP was recorded following treatment with the solution. Treatment with a composition according to Condition A or Condition B does not cause inflammation in animals.

[0281] No significant difference was observed between condition A and condition B.

[Table 12]: Average CRL levels measured in rats, expressed in $\mu\text{g/mL}$; D = day; NA = not applicable

		D-7	D-2	D2	D7	D28
Condition A	D7 sacrifice group	678.7 $\pm$ 172.8	502.2 $\pm$ 38.2	574.9 $\pm$ 43.7	432.7 $\pm$ 72.7	NA
	D28 sacrifice group	551.5 $\pm$ 68.0	482.2 $\pm$ 30.0	608.3 $\pm$ 102.1	438.5 $\pm$ 107.2	516.1 $\pm$ 107.7
Condition B	D7 sacrifice group	533.8 $\pm$ 58.3	513.4 $\pm$ 46.2	565.9 $\pm$ 48.4	456.0 $\pm$ 72.6	NA
	D28 sacrifice group	586.5 $\pm$ 116.7	605.8 $\pm$ 90.1	621.6 $\pm$ 115.6	546.3 $\pm$ 133.6	565.2 $\pm$ 99.3

[0282] No clinically relevant differences that can be attributed to treatment with the composition according to Condition A or according to Condition B were observed in the measured hematological and biochemical parameters. No significant difference was observed between condition A and condition B.

[0283] Pathomorphological analysis of the brains was performed 7 days and 28 days after treatment (two rats for each time point). No morphological changes indicative of toxicity in brain tissue were observed at 7 days or 28 days. No significant difference was observed between condition A and condition B.

[0284] No evidence of neurotoxicity was observed in the brain tissues examined. The microscopic findings, along with their severity grades, are depicted in Table 13.

- 5 [Table 13] Incidence of microscopic abnormalities in the brain 7 days and 28 days after treatment; D = day, Nb = number, 0 = no incidence; 1 = minimal incidence

	Group	Condition A	Condition B
D7 sacrifice	Number of animals examined	2	2
	Brain – Level 1 HES		
	Presence of foreign material	0	1
	Brain – Level 2 HES	0	0
	Brain – Level 1 MTG		
	Presence of foreign material	0	1
	Brain – Level 2 MTG	0	0
	Brain – Level 3 HE	0	0
D28 sacrifice	Brain – Level 4 HE	0	0
	Number of animals examined	2	2
	Brain – Level 1 HE		
	Presence of cellular debris (limited)	0	1
	Microglial center (limited)	0	1
	Brain – Level 2 HES		
	Presence of cellular debris (limited)	0	1
	Microglial center (limited)	0	1
	Brain – Level 1 MTG		
	Microglial center (limited)	0	1
	Brain – Level 2 MTG		
	Microglial center (limited)	0	1
	Brain – Level 3 HE	0	0
	Brain – Level 4 HE	0	0

- 10 [0285] In the brain tissue, minor microscopic abnormalities were observed in some animals in the treated groups on day 7 as well as on day 28. These findings are considered to be the result of minor trauma caused by the intracerebral injection procedure. The brain tissue samples examined showed no signs of neuronal toxicity.
- 15 [0286] No significant difference was observed between condition A and condition B.

[0287] In conclusion, treatment with the composition via intracerebral injections into the striatum under the experimental conditions of the study did not result in any signs of toxicity and was well tolerated. Consequently, this composition is biocompatible and does not cause toxicity, particularly when injected into the brain.

Example 6 - Effect of intracerebral injection of a composition on brain structure and inflammation in immunocompetent rats

[0288] To carry out this example, the composition used is solution (B) described in Example 1.

1. Protocol

a. Animal treatment

[0289] Immunocompetent heterozygous Rowett nude rats (Rnu-/+) were anesthetized using a 4% induction chamber. The rats were then placed under an anesthesia mask, using approximately 2.5% isoflurane.

[0290] Analgesia was achieved by subcutaneous injection of buprenorphine and lidocaine at concentrations of 0.05 mg/kg and 5 mg/kg, respectively. The rats were then injected with the composition into the right striatum using a 25- μ L Hamilton syringe with custom-made needles (glass cannula).

[0291] The left striatum was treated in the same manner but without injecting any composition (surgical control). The injection was performed using a stereotactic frame. At the end of the surgery, Metacam was administered subcutaneously at a concentration of 1 mg/kg for postoperative pain management.

b. Postmortem examination

1. Hematoxylin-eosin-saffron (HES) staining of brains and scoring of histological characteristics

5 [0292] The brains were collected 48 hours (7 samples), 1 week (6 samples), and 1 month (7 samples) after the procedure. The animals were first anesthetized by intraperitoneal injection of ketamine and xylazine.

[0293] The animals were then infused intracardially with 150 ml of 0.9% NaCl, followed by 200 ml of 10% phosphate-buffered formalin. After extraction, the
10 brains were post-fixed in 10% phosphate-buffered formalin for 24 hours at 4°C and finally stored in PBS at 4°C. After dehydration in successive baths of alcohol, acetone, and xylene, the samples were embedded in paraffin. 5-
15 µm slices (4–5 levels per sample) were prepared using a microtome and then mounted on treated slides using an albumin-glycerol mixture.

[0294] After deparaffinization, the sections were successively immersed in solutions of Harris's hematoxylin, eosin, and safranin. After dehydration, the sections were mounted between a slide and a coverslip using Entellan®.
20 Hematoxylin-eosin-safranin staining makes it possible to observe the morphology and structure of the tissues. The cytoplasm appears pink, and the nuclei appear blue-violet. The extracellular matrix was stained yellow to pink.

25 2. Masson-Goldner trichrome staining of brains stain and scoring of histological characteristics

[0295] After deparaffinization, the samples were fixed in Bouin's solution. The sections were successively immersed in Weigert's hematoxylin, acid fuchsin-Ponceau, phosphotungstic acid-orange G, and light green SF solutions. The
30 extracellular matrix appears green. The cytoplasm is stained pinkish-red, and the nuclei are stained blackish-purple.

[0296] Microscopic observations were performed using optical microscopy. Images were captured with a digital camera. One section per sample was selected for observation and for the semi-quantitative analysis of tissue lesions, inflammatory response, and residual bone fragments.

5

2. Results

[0297] After 48 hours, the right and left hemispheres of all the samples showed tissue changes characterized by localized hemorrhage (h) at the
10 injection site.

[0298] A marked but localized tissue lesion was observed in the nerve tissue (nv). No inflammatory reaction was observed, and only a few polymorphonuclear cells (PMNs) were detected, associated with
15 hemorrhagic infiltration. Randomly, residual fragments were observed only in the right hemisphere (three animals) or only in the left hemisphere (two animals). Most of the residual fragments were stained blue-green by the MTG stain, which is characteristic of extracellular matrix components and, more specifically, of bone (b). The observations are summarized in Table 14.

20

Table 14: 48 hours post-treatment: individual data from the semi-quantitative analysis of tissue lesion, inflammatory response, and residual bone fragments after treatment (right hemisphere) or no treatment (left hemisphere) with the solution.

25

Sample	Tissue lesion		Inflammatory response		Residual bone fragment	
Hemisphere	left	right	left	right	left	right
a	2	3	0	0	0	2
b	3	3	0	0	2	2
c	3	3	0	0	0	1
d	3	3	0	0	0	0
e	3	3	0	0	2	0
f	3	3	0	0	0	2
h	3	3	0	0	1	0
Average	2.86	3	0	0	0.71	1

- 0: no lesion/inflammatory reaction/residual bone fragments
 1: limited lesion/inflammatory reaction/residual bone fragments
 2: moderate lesion/inflammatory reaction/residual bone fragments
 3: significant lesion/inflammatory reaction/residual bone fragments
 5 4: severe lesion/inflammatory reaction/residual bone fragments

[0299] No differences in tissue lesion or inflammatory response were observed between the right hemisphere (treatment group) and the left hemisphere (control group). The density of the residual bone fragments varied depending on the sample.

[0300] One week after injection, the injection site was still visible upon macroscopic examination. At higher magnifications, residual bone fragments were still observable to varying degrees in the samples. A mild-to-moderate inflammatory response was observed in most samples, regardless of the hemisphere (and whether or not it was treated with the composition). Lymphocytes (l) and macrophages (m) were observed predominantly in the inflammatory infiltrates. Brown phagocytic granules were visible in the macrophages after HES staining.

[0301] The observations are summarized in Table 15 below.

[Table 15]: One week post-treatment: individual data from the semi-quantitative analysis of tissue lesion, inflammatory response, and residual bone fragments after treatment (right hemisphere) or without treatment (left hemisphere) with the solution.

Sample	Tissue lesion		Inflammatory response		Residual bone fragment	
Hemisphere	left	right	left	right	left	right
a	2	2	2	2	2	2
b	2	2	1	1	0	0
c	2	0	1	0	0	0
d	2	2	1	1	0	0
e	1	2	1	1	0	1

f	2	2	1	1	2	2
Average	1.83	1.67	1.17	1	0.67	0.83

0: no lesion/inflammatory reaction/residual bone fragments

1: limited lesion/inflammatory reaction/residual bone fragments

2: moderate lesion/inflammatory reaction/residual bone fragments

3: significant lesion/inflammatory reaction/residual bone fragments

5 4: severe lesion/inflammatory reaction/residual bone fragments

[0302] The results at one week post-treatment show no significant difference between the right hemisphere (solution) and the left hemisphere (control). An inflammatory reaction was noted when tissue lesion and residual bone fragments were also observed.

[0303] After one month, macroscopic observations at low magnification revealed no tissue lesions in the right and left hemispheres of any of the samples. At high magnification, moderate tissue lesion was observed in the right hemisphere, associated with the presence of residual bone fragments in three of the seven samples, but not associated with a tissue lesion in the left hemisphere.

[0304] As with the samples collected 48 hours and one week after treatment, the inflammatory response was limited and characterized by macrophages containing brown phagocytic granules.

[0305] The observations are summarized in Table 16 below.

[Table 16]: One month post-treatment: individual data from the semi-quantitative analysis of tissue lesion, inflammatory response, and residual bone fragments after treatment (right hemisphere) or without treatment (left hemisphere) with the solution

Sample	Tissue lesion		Inflammatory response		Residual bone fragment	
	left	right	left	right	left	right
a	0	1	0	1	0	0
b	0	0	0	0	0	0

c	0	2	0	1	0	2
d	1	2	1	2	0	0
e	1	1	1	1	0	0
f	0	2	0	1	0	1
h	0	2	0	1	0	2
Average	0.29	1.43	0.29	1	0	0.71

0: no lesion/inflammatory reaction/residual bone fragments

1: limited lesion/inflammatory reaction/residual bone fragments

2: moderate lesion/inflammatory reaction/residual bone fragments

3: significant lesion/inflammatory reaction/residual bone fragments

5 4: severe lesion/inflammatory reaction/residual bone fragments

[0306] Tissue lesions and inflammatory response were more pronounced in the right hemisphere, which had been injected with the composition, than in the left hemisphere (control). These observations were associated with the presence of residual bone fragments.

[0307] In conclusion, the injection site visible 48 hours after treatment disappears after one month. At 48 hours post-treatment, perforation of the nerve tissues was associated with significant bleeding. One week and one month after treatment, a reorganization of the nerve tissues was observed due to the persistence of bone debris deposited by the cannula during the injection. The pressure of the liquid injected into the right hemisphere was higher in the right hemisphere than in the left, where no liquid was injected; this may explain the presence of large amounts of residual bone fragments in the right hemisphere.

[0308] At these sites, a reaction to a foreign body was characterized by the presence of macrophages containing phagocytic granules. However, no acute inflammation was observed. Consequently, the composition used in this example does not cause inflammation when injected into the brain.

Example 7: Study of the viability of cellular elements in a solution comprising CMC.

[0309] The purpose of this example is to evaluate the viability of the cellular elements after immersion for 12 hours in a solution comprising CMC.

5 [0310] The various solutions used in this example are described in Table 17 below.

[Table 17]

	Dextran 40	CMC90	Sodium chloride	
M4	12.30%	4.60%	0.52%	
CM	Cellular elements cultured in a reference culture medium			
CS	Cellular elements cultured in a sodium chloride solution			

10 [0311] To carry out this example, the solution M4 was mixed with cellular elements in a controlled volume ratio of cellular elements to solution so as to form a composition according to the invention.

15 [0312] In the context of this example, the cellular elements consist of a mixture of microtissues having a largest dimension of at least 50 μm , isolated cells, and clusters of neuronal cells.

[0313] The composition according to the invention was kept at room temperature for 12 hours.

20

[0314] The cellular elements were then rinsed in an excess of reference culture medium. The microtissues were then collected, and viability was measured using a cell viability bioluminometer. The result was expressed as luminescence signals normalized by the CM control signal. To evaluate cell viability, seven analytical replicates were performed for each condition.

25

[0315] The results of this example show that the composition according to the invention, comprising the solution M4 and the cellular elements, maintains viability (96% with respect to a 12-hour control in a reference culture medium).

[0316] In conclusion, Example 7 demonstrates that the composition according to the invention, comprising the solution M4 and cellular elements, makes it possible to maintain high cell viability after immersion for 12 hours at room temperature.

Claims

- [Claim 1] A biocompatible composition, comprising a solution and cellular elements suspended in said solution, said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and mixtures thereof, at least two of said cellular elements having different density and/or size and/or cell count, said composition comprising at least:
- hyaluronic acid and/or carboxymethyl cellulose (CMC), and
 - at least one biocompatible density agent,
 - and/or at least one modified form of one or more of these molecules.

[Claim 2] The composition according to the preceding claim, characterized in that at least one cellular element is a microtissue having a largest dimension of at least 50 μm .

[Claim 3] The composition according to one of the preceding claims, characterized in that at least one biocompatible densifying agent has a molar mass greater than 400 g/mol.

[Claim 4] The composition according to one of the preceding claims, characterized in that at least one biocompatible density agent is selected from dextran, hypromellose, iodixanol, polyethylene glycol (PEG), chitosan, pullulan, or molecules of the glycosaminoglycan family, or modified forms of these molecules, and mixtures thereof.

[Claim 5] The biocompatible composition according to one of the preceding claims, characterized in that the cells constituting the cellular elements are selected from neural cells, neuronal cells, glial cells, and mixtures thereof.

[Claim 6] The biocompatible composition according to one of the preceding claims, characterized in that at least one cell constituting the cellular elements is a dopaminergic neuron.

[Claim 7] The biocompatible composition according to one of the preceding claims, characterized in that the cells constituting the cellular elements belong to at least two distinct cell types.

5

[Claim 8] The biocompatible composition according to one of the preceding claims, characterized in that the cells constituting the cellular elements comprise astrocytic cells, oligodendrocytic cells, and dopaminergic neurons.

10 [Claim 9] The biocompatible composition according to one of the preceding claims, characterized in that it comprises at least one cellular element contained in a three-dimensional microcompartment comprising an outer hydrogel layer.

[Claim 10] The biocompatible composition according to one of the preceding
15 claims, characterized in that the volume fraction of the cellular elements is between 0.01 and 80% by volume.

[Claim 11] The biocompatible composition according to one of the preceding claims, characterized in that it comprises:

- 20 - sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa and/or carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa; and/or
- dextran having a molecular weight of between 40 and 80 kDa.

25 [Claim 12] The composition according to one of the preceding claims, characterized in that it comprises a solution comprising at least one ion selected from sodium ions, calcium ions, chloride ions, potassium ions, and mixtures thereof.

30 [Claim 13] The composition according to one of the preceding claims, characterized in that it comprises a solution comprising at least one element

selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

[Claim 14] The biocompatible composition according to one of the preceding claims, characterized in that the composition comprises:

- between 0.1 and 7% by mass of sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa, with respect to the total mass of the solution, and/or of between 0.1 and 7% by mass of carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa, with respect to the total mass of the solution; and

- between 10 and 25% by mass of at least one density agent with respect to the total mass of the solution; and

- between 0.01 and 0.9% by mass of NaCl with respect to the total mass of the solution; and/or

- between 0.01 and 0.04% by mass of KCl with respect to the total mass of the solution; and/or

- between 0.01 and 0.05% by mass of CaCl₂ with respect to the total mass of the solution.

[Claim 15] The biocompatible composition according to one of the preceding claims, characterized in that the composition has a density of between 800 and 2000 kg/m³.

[Claim 16] The biocompatible composition according to one of the preceding claims, characterized in that the composition has a static viscosity, measured using a rheometer at 25°C, of between 0.01 and 1.5 Pa·s.

[Claim 17] The biocompatible composition according to one of the preceding claims, characterized in that the osmolality of the solution is between 180 and 360 mOsmol·kg⁻¹.

[Claim 18] The biocompatible composition according to one of the preceding claims, characterized in that the maximum sedimentation rate of the cellular elements is between 0.1 and 20 mm·h⁻¹.

- 5 [Claim 19] The composition according to one of the preceding claims, for use in the treatment of diseases by cell therapy.

[Claim 20] The composition for use according to the preceding claim, in the treatment of Parkinson's disease.

10

[Claim 21] The composition for use according to one of claims 19 or 20, by injection using a cannula into the putamen in humans or animals.

- 15 [Claim 22] The composition according to one of the preceding claims, characterized in that it is obtained by carrying out the following steps:

(a) Preparing or recovering a composition comprising a set of cellular elements, at least two cellular elements of which have a different density and/or size and/or cell count;

(b) Preparing a solution comprising:

- 20 - hyaluronic acid and/or carboxymethyl cellulose (CMC), and

- at least one biocompatible density agent,

- and/or at least one modified form of one or more of these molecules;

(c) Sterilizing the solution resulting from step (b) so as to obtain a sterilized filtrate;

(d) Mixing the cellular elements resulting from step (a) with the sterilized

- 25 filtrate resulting from step (c);

(e) Optionally recovering the biocompatible composition resulting from step (d), comprising a solution and cellular elements suspended in said solution, at least two cellular elements of which have a different density and/or size and/or cell count, said composition comprising the following molecules:

- 30 - Hyaluronic acid and/or carboxymethyl cellulose (CMC);

- At least one biocompatible density agent;

- and/or at least one modified form of one or more of these molecules.

[Claim 23] A method for preparing a biocompatible composition according to one of the preceding claims, characterized in that it comprises carrying out the following steps:

- 5 (a) Preparing or recovering a composition comprising a set of cellular elements, at least two cellular elements of which have a different density and/or size and/or cell count;
- (b) Preparing a solution comprising:
 - hyaluronic acid and/or carboxymethyl cellulose (CMC), and
 - 10 - at least one biocompatible density agent,
 - and/or at least one modified form of one or more of these molecules;
- (c) Sterilizing the solution resulting from step (b) so as to obtain a sterilized filtrate;
- (d) Mixing the cellular elements resulting from step (a) with the sterilized filtrate resulting from step (c);
- 15 (e) Optionally recovering the biocompatible composition resulting from step (d), comprising a solution and cellular elements suspended in said solution, at least two cellular elements of which have a different density and/or size and/or cell count, said composition comprising the following molecules:
 - Hyaluronic acid and/or carboxymethyl cellulose (CMC);
 - 20 - At least one biocompatible density agent;
 - and/or at least one modified form of one or more of these molecules.

[Claim 24] The method according to the preceding claim, characterized in that step (a) comprises carrying out the following steps:

- 25 1) Culturing at least one cellular element;
- 2) Rinsing the culture of cellular elements resulting from step 1);
- 3) Centrifuging the culture of cellular elements resulting from step 2);
- 4) Recovering a composition comprising cellular elements resulting from the centrifugation of step 3), at least two cellular elements of which have a
- 30 different density and/or size and/or cell count.

[Claim 25] The method according to the preceding claim, characterized in that at least one cellular element comprises at least one encapsulated cell, preferentially encapsulated in alginate.

- 5 [Claim 26] The method according to one of claims 23 to 25, characterized in that the composition of step (a) comprises a solution in which said cellular elements are suspended.

[Claim 27] The method according to one of claims 23 to 26, characterized in
10 that the composition of step (a) has a maximum sedimentation rate of the cellular elements with respect to the solution greater than $20 \text{ mm} \cdot \text{h}^{-1}$.

[Claim 28] The method according to one of claims 23 to 27, characterized in that the solution of step (b) comprises:

- 15 - between 0.1 and 7% by mass of sodium hyaluronate having a molecular weight of between 80 kDa and 1 MDa, with respect to the total mass of the solution, and/or of between 0.1 and 7% by mass of carboxymethyl cellulose having a molecular weight of between 40 kDa and 1 MDa, with respect to the total mass of the solution; and
20 - between 10 and 25% by mass of at least one biocompatible density agent with respect to the total mass of the solution.

[Claim 29] The method according to one of claims 23 to 28, characterized in that step (b) further comprises adding at least one element selected from
25 sodium chloride (NaCl), calcium chloride (CaCl_2), potassium chloride (KCl), magnesium chloride (MgCl_2), and mixtures thereof.

[Claim 30] The method according to the preceding claim, characterized in that the solution resulting from step (b) likewise comprises:

- 30 - between 0.01 and 0.9% by mass of NaCl with respect to the total mass of the solution; and/or

- between 0.01 and 0.04% by mass of KCl with respect to the total mass of the solution; and/or
- between 0.01 and 0.05% by mass of CaCl_2 with respect to the total mass of the solution.

5

[Claim 31] The method according to one of claims 23 to 30, characterized in that it comprises a step (c'), carried out before step (c), of adjusting the pH of the solution resulting from step (b), so that the solution resulting from step (c') has a pH of between 6.8 and 8.

10

[Claim 32] The method according to one of claims 23 to 31, characterized in that step (d) is carried out with stirring.

[Claim 33] A kit comprising:

- 15
- a biocompatible composition according to one of claims 1 to 22; and
 - an injection means, preferentially a cannula.

[Claim 34] The kit according to the preceding claim, characterized in that the biocompatible composition is in solid form.

20

[Claim 35] A kit comprising:

- A preparation, in solid form, comprising the following molecules:
 - Hyaluronic acid and/or carboxymethyl cellulose (CMC); and at least one density agent and/or a modified form of these molecules;
- 25 - A preparation comprising cellular elements, said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and mixtures thereof, at least two of said cellular elements having a different density and/or size and/or cell count.

30 [Claim 36] The kit according to one of claims 33 to 34, characterized in that it comprises water.

[Claim 37] The kit according to one of claims 33 to 35, characterized in that it comprises at least one element selected from sodium chloride (NaCl), calcium chloride (CaCl_2), potassium chloride (KCl), magnesium chloride (MgCl_2), and mixtures thereof.

5

[Claim 38] The kit according to one of claims 33 to 37, characterized in that one or more cells constituting the cellular elements are selected from neuronal cells, glial cells, and a mixture thereof.