

BIOCOMPATIBLE COMPOSITION COMPRISING HETEROGENEOUS CELLULAR ELEMENTS

TECHNICAL FIELD

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[0001] The present invention relates to the field of biocompatible compositions, particularly injectable biocompatible compositions. In particular, the invention relates to a particular biocompatible composition comprising a solution and heterogeneous cellular elements suspended in said solution.

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BACKGROUND ART

[0002] For more than 20 years, regenerative medicine—and cell therapy in particular—has been attracting growing interest among healthcare professionals and the general public. Cell therapy involves transplanting cells to restore the function of a tissue or organ by injecting therapeutic cells.

[0003] Today, cell therapy faces many challenges inherent in the administration of cells into the body.

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[0004] 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 administered is a fundamental challenge for any injectable therapy.

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[0005] In general, medicaments can be dissolved in a liquid and administered as a solution, such as through an IV drip. Liquid suspensions comprising very small particles also behave like solutions and can also be administered by infusion.

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[0006] Conversely, once the composition is cellularized—i.e., once it contains cells—it is injected as a suspension.

[0007] Unlike solutions that form a homogeneous, fluid liquid, suspended cells naturally settle over time.

5 [0008] For example, “normal” red blood cells settle in the blood at a relative velocity of up to 20 mm per hour, and when certain cells are aggregated into clumps or microtissues, this relative velocity can exceed 100 mm per hour, or even 300 mm per hour in some cases.

10 [0009] Particle sedimentation in solid-liquid suspensions is a topic widely discussed in many fields, such as chemistry, geology, and biology, particularly in pharmaceutical applications and bioprinting.

15 [0010] The sedimentation velocity of solid particles, such as cells, in a liquid refers to the velocity at which the particles move downward or settle to the bottom of a container due to gravity. This sedimentation phenomenon occurs when particles, due to their mass and density, are heavier than the medium in which they are suspended.

20 [0011] When using a cell suspension, the cells may be heterogeneous. In fact, cells can take on a wide variety of forms; these are referred to as cellular elements. For example, cellular elements may take the form of isolated cells, cellular microtissues, or cell clusters having a different density and/or size and/or number of cells. As a result, when using suspensions containing
25 heterogeneous cellular elements, the cells are distributed heterogeneously within their medium based on several parameters, such as the size, shape, and density of the cells, as well as the physicochemical properties of the medium.

30 [0012] Without controlling these parameters, the highest concentration of cells accumulates, due to gravity, in the lower part of the device comprising the suspension of cellular elements.

[0013] For example, in cell therapy, when injecting a suspension comprising heterogeneous cellular elements, the first injection volume may contain many more cells than those delivered subsequently. This density gradient creates
5 heterogeneity within the various injection volumes, which can compromise the effectiveness of the cell therapy.

[0014] Furthermore, in addition to maintaining a uniform distribution of cells within their medium, maintaining biological stability is essential to ensuring the
10 survival of living cells in that medium, particularly when injecting viable cells for use in cell therapy. In fact, osmolality, concentration, and the diversity of ionic species are essential for maintaining the homeostasis of cells in suspension.

[0015] There is therefore a significant need for a biocompatible composition, comprising heterogeneous cellular elements, capable of maintaining:
15 - the uniform distribution of cellular elements within the composition, so as to be able to precisely control the number of cells contained in a given volume; and
20 - the biological stability of suspended cellular elements.

SUMMARY OF THE INVENTION

[0016] To address this need, the invention proposes a new biocompatible
25 composition comprising a solution and cellular elements suspended in said solution, which cellular elements are 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 number of cells, said composition being characterized in that the difference in density between the
30 density of the solution and the average density of the cellular elements satisfies the following inequality:

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

- 5 where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,
where D represents the largest dimension of the cellular elements, expressed
10 in meters,
where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;
where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to
15 gravity expressed in $\text{m}\cdot\text{s}^{-2}$;
and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

- [0017] Advantageously, satisfying the aforementioned inequality ensures that the maximum sedimentation velocity of the cellular elements with respect to
20 the solution is less than $5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$, or $20 \text{ mm}\cdot\text{h}^{-1}$.

- [0018] The maximum sedimentation velocity 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:

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- [0019] - Increased cell viability: A maximum sedimentation velocity of less than $20 \text{ mm}\cdot\text{h}^{-1}$ reduces the mechanical stress exerted on the cellular elements, thereby minimizing the risk of damage to their structure or metabolism, which can improve their viability;

- Increased analytical precision: For specific analyses and measurements, a maximum sedimentation velocity of less than $20 \text{ mm} \cdot \text{h}^{-1}$ results in greater precision and sensitivity in the results;
- improved homogeneity: A maximum sedimentation velocity of less than $20 \text{ mm} \cdot \text{h}^{-1}$ ensures that the cells remain evenly distributed throughout the solution, thereby preventing the formation of concentration gradients;
- improved experimental reproducibility: By maintaining a maximum sedimentation velocity below $20 \text{ mm} \cdot \text{h}^{-1}$, variations caused by the differential sedimentation of heterogeneous cellular elements are reduced.

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[0020] The composition according to the invention has a density and/or viscosity that is particularly well-suited to heterogeneous cellular elements, promoting a uniform distribution of said cellular elements in suspension in the solution.

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[0021] 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 $360 \text{ mOsmol} \cdot \text{kg}^{-1}$, preferably between 220 and $360 \text{ mOsmol} \cdot \text{kg}^{-1}$. In other words, the composition comprising cellular elements and a solution has an osmolality of between 180 and $360 \text{ mOsmol} \cdot \text{kg}^{-1}$, preferably between 220 and $360 \text{ mOsmol} \cdot \text{kg}^{-1}$.

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[0022] Advantageously, the osmolality of the cellular elements helps create an environment conducive to their survival, such as an isotonic environment.

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[0023] Surprisingly, the inventors have developed a biocompatible composition suitable for a wide range of applications that controls the sedimentation velocity of heterogeneous cellular elements suspended in the solution while exhibiting biological stability suitable for cells, particularly human or animal cells.

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[0024] Preferably, the composition according to the invention has a density between 800 and 2000 kg/m³, more preferably between 997 and 1600 kg/m³, and most preferably between 1025 and 1100 kg/m³, specifically between 1047 and 1090 kg/m³.

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[0025] Advantageously, the density of the composition according to the invention is homogeneous; as a result, each cellular element is subjected to the same density throughout the entire volume of the composition.

10 [0026] According to a preferred embodiment, the composition according to the invention has a resting viscosity measured at 25°C ranging from 0.01 to 1.5 Pa·s, preferably ranging from 0.01 to 1 Pa·s, more particularly ranging from 400 to 1200 mPa·s, more preferably between 400 and 1000 mPa·s, and even more preferably between 500 and 1100 mPa·s, in particular between 500 and
15 900 mPa·s.

[0027] Advantageously, 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
20 encountered during injection impairing the precision of the injection or affecting the viability and integrity of the cells. In fact, excessive viscosity and/or density can lead to increased resistance as the composition flows through the injection means, combined with viscous friction forces that may damage the cellular elements.

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[0028] The inventors have developed a biocompatible composition exhibiting rheological properties suited to heterogeneous cellular elements by taking into account the density-viscosity relationship of the solution comprising said cellular elements.

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[0029] According to one embodiment, the biocompatible composition comprises at least one cellular element contained within a three-dimensional microcompartment comprising an outer hydrogel layer.

- 5 [0030] Preferably, the biocompatible composition according to the invention 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, cardiac cells, retinal cells, lymphocytes, hepatocytes, endothelial cells, mesenchymal stem cells, keratinocytes,
10 fibroblasts, pre-adipocytes, adipocytes, pancreatic islet cells, and mixtures thereof.

[0031] According to one embodiment, the composition according to the invention comprises at least two distinct cell types.

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[0032] Advantageously, the composition according to the invention is particularly well-suited for all cell types.

- [0033] According to another aspect, the invention relates to the biocompatible
20 composition according to the invention for use in the treatment of diseases through cell therapy.

[0034] Preferably, the biocompatible composition according to the invention is used as an injectable composition in humans or animals.

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[0035] Advantageously, by ensuring uniform distribution of the cellular elements within the solution, the composition according to the invention allows for precise dosing of the injected cellular elements, thereby improving:

- 30 [0036] - Therapeutic efficacy: When injecting cellular elements for therapeutic purposes, precise dosing is essential to achieve the desired result. An insufficient amount of cellular elements may not be effective, while an

overdose of cellular elements may cause adverse effects. By precisely adjusting the dosage, it is possible to maximize therapeutic efficacy while minimizing risks to the patient;

- 5 [0037] - Experimental precision: Controlling the dosage during the injection of cellular elements helps minimize variations between samples and ensures reliable and reproducible data. This is particularly important in comparative studies or tests of the efficacy of new cell therapies.
- 10 [0038] - Resource optimization: The resources used to produce cellular elements, such as cell cultures and cell culture reagents, can be costly. Accurate dosing helps minimize waste of these resources by avoiding unnecessary overdoses.
- 15 [0039] The invention also relates to a method for preparing a biocompatible composition according to the invention comprising carrying out the following steps:
- [0040] (a) Preparation or recovery of a composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density and/or size and/or number of cells;
- 20 [0041] (b) Preparation of a biocompatible composition comprising the cellular elements of the composition from step (a), solution (B), and optionally solution (A), such that the difference in density between the density of the solution of the composition obtained in step (b) and the average density of the cellular elements of the composition obtained in step (b) satisfies the following inequality:
- 25 [Math 1]
- 30 [Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m³, and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m³,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in m·s⁻¹;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in Pa·s, where g represents the acceleration due to gravity expressed in m·s⁻²;

and where $v_c = 5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$;

(c) Optionally, recovery of the composition obtained in step (b), comprising a solution and cellular elements suspended in said solution, at least two of which cellular elements have a different density and/or size and/or number of cells, wherein said composition is characterized in that the difference in density between the density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b).

[0042] 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 keeping the heterogeneous cellular elements in suspension at a maximum sedimentation velocity with respect to the solution of less than $5.56 \cdot 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or $20 \text{ mm} \cdot \text{h}^{-1}$.

[0043] Preferably, the composition of step (a) has a maximum sedimentation velocity of the cellular elements with respect to solution (A) greater than $20 \text{ mm} \cdot \text{h}^{-1}$.

[0044] Such a method is particularly useful for adjusting the rheological properties—specifically the density and viscosity—of a composition so as to ensure a homogeneous distribution of the heterogeneous cellular elements within the composition. In this way, the composition from step (c) can be used
5 in many applications, particularly in cell therapy.

[0045] Finally, according to a last object, the invention relates to a kit comprising:

- a biocompatible composition comprising a solution and cellular elements
10 suspended in said solution, which cellular elements are 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 number of cells, said composition being characterized in that the difference in density between the density of the solution and the average density of the cellular
15 elements satisfies the following inequality:

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

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where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m³, and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m³,

25 where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in m·s⁻¹;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity
30 of the solution expressed in Pa·s, where g represents the acceleration due to gravity expressed in m·s⁻²;

and where $v_c = 5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$; and

- an injection means, preferably a cannula.

[0046] Advantageously, the rheological properties of the composition make it possible to maintain a uniform distribution of cellular elements throughout the composition while preventing blockage of the injection means.

[0047] According to an alternative, the invention relates to a kit comprising:

- a biocompatible preparation (1) comprising at least one biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl cellulose (CMC), methyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, hydroxypropyl methylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide, polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules; and at least one biocompatible density-adjusting agent selected from dextran, mannitol, NaCl, glycerol, sorbitol, and mixtures thereof; and
 - a biocompatible preparation (2) 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 number of cells
- so that the mixture of preparation (1) and preparation (2) forms a composition in which the difference in density between the density of preparation (1) and the average density of the cellular elements of preparation (2) satisfies the following inequality:

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

5 where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18.\mu_c/g \sim 9.81$, with μ_c representing the maximum static viscosity
10 of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

and where $v_c := 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

[0048] Further features and advantages will become apparent from the
15 detailed description of the invention and the examples and figures that follow.

BRIEF DESCRIPTION OF THE FIGURES

[0049] [Figure 1] Figure 1 shows a sequence of images showing the stability
20 of cellular elements in suspension over time in a composition according to the invention and a composition outside the scope of the invention.

[0050] [Figure 2] Figure 2 shows a comparative study of the impact of
prolonged immersion (6 hours) of cellular elements in a solution on a selection
25 of production batches of the microtissue of interest.

[0051] [Figure 3] Figure 3 is a schematic representation of a comparative study
evaluating the impact of an injection procedure on the viability of cellular
elements within the composition according to the invention.

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[0052] [Figure 4] Figure 4 is a pictorial of a study evaluating the various shear strains of the composition according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

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Definitions

[0053] For the purposes of the invention, the term “density-adjusting agent” refers to a biocompatible chemical compound that is soluble in aqueous solution and is used to increase the density of that solution without significantly altering the solution’s biocompatibility with the suspended cellular elements. More specifically, when a density-adjusting agent as defined by the invention is added to an aqueous solution at a concentration greater than 10% by mass, this does not cause any change in the solution’s rheological behavior.

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15 Furthermore, adding such an agent does not increase the osmolality of the solution beyond 360 mOsm. In other words, adding a density-adjusting 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.

20 [0054] For the purposes 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.

[0055] For the purposes 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, it means that it is designed and formulated to minimize the risk of toxicity, inflammation, rejection, or other adverse reactions by the body and/or cellular elements.

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[0056] 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 according to the invention are constituted by or are obtained from human cells
5 or from immunologically humanized non-human mammalian cells.

[0057] For the purpose of the invention, "progenitor cell" refers to a stem cell already engaged in cellular differentiation but not yet differentiated.

10 [0058] For the purposes 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 can be assessed by the presence of markers such as the transcription factors OCT4, NANOG, and SOX2, and surface markers such as SSEA4/5, Tra-1-60, and Tra-1-81. The embryonic
15 stem cells used in 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.

20 [0059] 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
25 pluripotent stem cells (iPSC or hiPSC for human induced pluripotent stem cells), embryonic stem cells or MUSE cells (for "Multilineage-differentiating Stress Enduring").

[0060] For the purpose of the invention, "induced pluripotent stem cell" refers
30 to a pluripotent stem cell induced to pluripotency by genetic reprogramming of differentiated somatic cells. These cells are notably positive for 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, 2007, 131(5): 861-872) and Nakagawa et al (Nat Biotechnol, 2008, 26(1): 101-106).

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[0061] For the purposes of the invention, the term “rheological behavior” refers to a material’s response to the stresses and strains applied to it. It describes how matter flows or deforms under the influence of an external force. There are several types of rheological behavior, such as elasticity, plasticity, viscosity, and viscoelasticity.

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[0062] 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. This layer is organized in three dimensions in the microcompartment.

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[0063] For the purposes of the invention, the term “density” refers to the density of the entities in question (cellular elements or solutions), expressed in mg/mL.

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In the context of the invention, the density of the solutions is measured at a temperature of 20°C using a hydrometer. For cellular elements, density is measured using the density gradient method. This involves 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 gradients are prepared in 5 kg/m³ increments. The density of the cellular elements is therefore given with a resolution of +/- 5 kg/m³. Once the density gradient has formed, the cellular elements are injected into the upper portion of the tube. These particles settle and stabilize within or between the layer(s) whose density is closest to theirs. The reading is taken once all cellular elements have stabilized, typically after 1 hour. Advantageously, colored synthetic beads of calibrated densities can be

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introduced simultaneously, with their equilibrium position serving as a positive control for the position measurement of the cellular elements. All of these measurements are taken at the operating temperature or within the projected operating temperature range, since temperature can affect the density of water, solutions, and cellular elements in different ways.

[0064] For the purposes of the invention, the term “average density of the cellular elements” refers to the average density of the cellular elements such as microcompartments. In the context of the invention, the average density of the cellular elements is measured using the density gradient method. This method, which is well known to those in the field, involves 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, with each layer being denser than the layer directly above it. Once the density gradient has formed in the tube, the cellular elements are introduced into the tube and settle, stabilizing in or between the layers whose density is closest to their own. The reading is taken once all cellular elements have stabilized, typically after 1 hour. Along with the cellular elements, colored synthetic beads of known density are also introduced into the tube to serve as a positive control. The average density is then calculated based on the distribution of the cellular elements within the tube.

[0065] For the purposes of the invention, “Feret diameter” of a cellular element refers to the distance between two tangents, which two tangents are parallel, such that the entire cellular element lies between these two parallel tangents.

[0066] 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.

[0067] For the purposes 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 or not, and mixtures thereof. Microtissues and/or clusters, whether encapsulated or not, may contain one or more lumens.

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[0068] For the purposes 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 molecules. For example, hyaluronic acid consists of a chain of monomers,
10 each of which contains hydroxyl, carboxyl, and N-acetyl functional groups that can serve as attachment sites for molecular groups. Hydroxyl and carboxyl groups are preferred sites for the attachment of lipids, polymers, or other active molecules to the hyaluronic acid monomer. In addition, amine groups can also be added to the N-acetyl functional group. This makes it possible to create
15 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 can participate in a wide variety of addition reactions (hydroxyl group chemistry).

20 [0069] For the purpose of the invention, “largest dimension” refers to the value of the largest Feret diameter. The dimensions of the cellular elements can be determined using various techniques. In particular, it can be characterized using a Coulter-type measuring device. Alternatively, it can be determined optically using microscopy.

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[0070] For the purposes of the invention, “keeping the cellular elements in suspension” refers to slowing the sedimentation velocity of the cellular elements without affecting their survival.

30 [0071] For the purpose of the invention, “microcompartment” or “capsule” also refers to a partially or totally enclosed three-dimensional structure containing one or more cellular elements. The structure preferably consists of a stiffened

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.

5 [0072] For the purposes 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 microtissue exhibits a specific topology in the distribution of its constituent
10 cells. For the purposes of the invention, a microtissue has a largest dimension of at least 50 μm , more preferably between 50 μm and 1.3 mm, and in particular between 100 μm and 800 μm .

[0073] For the purposes of the invention, “osmolality of cellular elements”
15 refers to a measurement of the total concentration of dissolved particles within cellular elements, expressed in osmoles per kilogram of water ($\text{osmol/kg H}_2\text{O}$).

[0074] It includes active solutes, such as electrolytes and nonionic molecules. In the context of the invention, osmolality can be measured using a freezing-
20 point osmometer such as the “Osmotech XT.” It should be noted that using different models of freezing-point osmometers may make measurement impossible. In fact, the composition of the solutions and their viscosity make measuring osmolality a complex task and require high-powered instruments. Alternatively, osmolality can be calculated by extrapolation based on a
25 measurement of a dilution of the solution, which allows for the use of standard instruments.

[0075] For the purposes of the invention, the term “active ingredient” refers to one or more substances responsible for a pharmacological or therapeutic
30 effect.

[0076] For the purposes of the invention, the term "biological stability" refers to the maintenance of the integrity of the cellular elements.

[0077] For the purpose of the invention, "tissue" or "biological tissue" refers to
5 the common meaning of tissue in biology, i.e. the intermediate level of 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
10 work together to perform the same function. Biological tissues regenerate regularly and are joined together to form organs.

[0078] For the purposes of the invention, the term "sedimentation velocity threshold" or "maximum sedimentation velocity" refers to the maximum
15 measured relative velocity of vertical displacement within the solution relative to the reference frame of the system, container, or fluidic element in which the suspension is contained. The maximum relative velocity is expressed in mm/h. By convention, relative velocity is defined as positive when the cellular elements settle in the solution, and negative when they rise in the solution. The
20 term "maximum" refers to the absolute value of the velocity, regardless of its sign, in the sense that, in the context of the invention, it is important that the cellular elements remain in suspension, which implies that their relative velocity should be as low as possible in absolute terms.

[0079] For the purposes of the invention, the term "viscosity" refers to dynamic
25 viscosity, which characterizes a fluid's resistance 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 whose viscosity decreases with shear rate.
30 Similarly, a fluid is called a "yield-stress fluid" if its viscosity is very high at low shear rates but drops sharply once the shear rate exceeds a certain threshold value. These two categories of fluids are advantageous in the context of the

invention because their high resting viscosity prevents sedimentation, while fluidization at high shear rates facilitates the flow of the solution and limits viscous friction forces on biological elements, for example during injection. In the context of the invention, viscosity can be measured at 25°C using a
5 rheometer via the shear-viscosity curve method, with the fluid confined within a 25-mm-diameter Cone-Plate geometry, at an angle of 1°, with a sandblasted surface, and over a range of shear rates from $\dot{\gamma} = 0.1 \text{ s}^{-1}$ to $\dot{\gamma} = 1000 \text{ s}^{-1}$.

[0080] "Resting viscosity" refers to the dynamic viscosity measured at rest, that
10 is, at a very low shear rate $\dot{\gamma}$ on the fluid, typically $\dot{\gamma} = 0.1 \text{ s}^{-1}$.

[0081] For the purposes of the invention, "viability" refers to the proportion of healthy cells in a sample of cellular elements. In the context of the invention, the proportion of healthy cells is assessed based on the metabolic activity of a
15 cell sample. Metabolic activity is quantified by measuring the ATP concentration assayed in a suspension of lysed cellular elements. The technique used is marketed under the name "CellTiter-Glo® 3D Cell Viability Assay." It is specifically designed to determine cell viability in 3D microtissue spheroids. The test reagent penetrates large spheroids and has increased lytic
20 capacity, allowing for a more accurate determination of viability with respect to other testing methods. Preferably, viability is expressed as a percentage with respect to a control condition. In this case, viability is referred to as "relative." For example, if condition A is taken as a reference, a viability of 85% for condition B represents a 15% decrease in viability with respect to condition A.

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Biocompatible composition according to the invention

[0082] The present invention relates to a biocompatible composition comprising a solution and cellular elements suspended in said solution, which
30 cellular elements are 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 number of cells, and the difference in

density between the density of the solution and the average density of the cellular elements satisfies the following inequality:

[Math 1]

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$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m³, and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m³,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in m·s⁻¹;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in Pa·s, where g represents the acceleration due to gravity expressed in m·s⁻²;

and where $v_c = 5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$.

20

[0083] Surprisingly, the inventors have developed a biocompatible composition capable of keeping heterogeneous cellular elements in suspension while maintaining their biological stability over a long period of time. Thus, the rheological properties of the solution advantageously help maintain a uniform distribution of the cellular elements within the composition.

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[0084] According to one embodiment, the sedimentation velocity threshold is less than $2.78 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or 10 mm·h⁻¹.

30

[0085] According to another embodiment, the acceptable sedimentation velocity threshold is less than $1.39 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or 5 mm·h⁻¹.

[0086] According to another embodiment, the acceptable sedimentation velocity threshold is less than $2.78 \times 10^{-7} \text{ m}\cdot\text{s}^{-1}$, or $1 \text{ mm}\cdot\text{h}^{-1}$.

- 5 [0087] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the maximum sedimentation velocity of the cellular elements with respect to the solution is between 2.78×10^{-9} and $5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$, or between 0.01 and $20 \text{ mm}\cdot\text{h}^{-1}$.

10

[0088] Thus, the lower the maximum sedimentation velocity of the cellular elements, the better the homogeneity of the distribution of the cellular elements in suspension within the solution is maintained over time.

- 15 [0089] Preferably, the osmolality of cellular elements in the solution is between 180 and $360 \text{ mOsm}\cdot\text{kg}^{-1}$, and even more preferably between 220 and $360 \text{ mOsm}\cdot\text{kg}^{-1}$. 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.

20

[0090] Preferably, the composition according to the invention comprises a solution comprising at least one ion selected from sodium, calcium, magnesium, chlorine, and potassium ions and mixtures thereof.

- 25 [0091] According to a preferred embodiment, the composition according to the invention comprises a solution comprising sodium, calcium, chlorine, and potassium ions.

- [0092] Preferably, the composition according to the invention comprises a
30 solution and cellular elements suspended in said solution, wherein the solution is an aqueous solution.

[0093] According to one embodiment, the composition comprises a solution and cellular elements suspended in said solution, wherein the solution has a pH between 7.0 and 7.6.

- 5 [0094] Advantageously, the pH of the solution is particularly well-suited to the survival of cellular elements.

[0095] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said
10 solution, wherein the solution comprises a biocompatible pH buffer, preferably selected from phosphate-buffered saline (PBS), citric acid or HEPES, and preferably the PBS buffer listed under number 4004200 in the European Pharmacopoeia.

- 15 [0096] According to one alternative, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution also comprises at least one active ingredient and/or a pharmaceutically acceptable excipient.

- 20 [0097] According to a specific embodiment of the invention, the aqueous solution serves as the culture medium for the cellular elements.

[0098] According to another embodiment, the composition according to the invention comprises a solution comprising at least one element selected from
25 sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

[0099] Advantageously, the diversity of ions contained in the solution helps ensure a balanced environment conducive to the proper functioning of cellular
30 processes in the cellular elements within the composition according to the invention.

[0100] Preferably, 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

5 - 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.

10 [0101] 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 the risk of osmotic shock.

[0102] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution
15 is isotonic with respect to the cellular elements.

[0103] According to one embodiment, the composition according to the invention has a density between 800 and 2000 kg/m^3 , preferably between 997 and 1600 kg/m^3 , more preferably between 1025 and 1100 kg/m^3 , specifically
20 between 1047 and 1090 kg/m^3 .

[0104] Advantageously, the density of the composition according to the invention contributes to a reduction in the maximum sedimentation velocity of the cellular elements with respect to the solution.
25

[0105] According to one alternative, 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 with the lowest density and the density of the cellular element with the highest density.
30

[0106] According to another alternative, 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 with the lowest density.

5 [0107] Finally, according to another alternative, 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 with the highest density.

10 [0108] Advantageously, in all embodiments according to the invention, the maximum sedimentation velocity of the cellular elements with respect to the solution is less than $5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$, or $20 \text{ mm}\cdot\text{h}^{-1}$.

15 [0109] According to another alternative, the composition according to the invention has a density equal to the average of the densities of all cellular elements present in the composition, with a permissible variation of 20 kg/m^3 , preferably 10 kg/m^3 . In this way, the density of the composition is centered on the average density of all the cellular elements.

20 [0110] According to a preferred embodiment, the composition according to the invention has a resting viscosity measured at 25°C ranging from 0.01 to $1.5 \text{ Pa}\cdot\text{s}$, preferably ranging from 0.01 to $1 \text{ Pa}\cdot\text{s}$, more particularly ranging from 400 to $1200 \text{ mPa}\cdot\text{s}$, more preferably between 400 and $1000 \text{ mPa}\cdot\text{s}$, and even more preferably between 500 and $1100 \text{ mPa}\cdot\text{s}$, in particular between 500 and $900 \text{ mPa}\cdot\text{s}$.

25

[0111] The viscosity of the composition prevents the cellular elements suspended in the solution from settling, while allowing the composition according to the invention to be used, in particular by injecting said composition. As a result, the composition according to the invention has a
30 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.

5 [0112] According to a specific alternative of the invention, the biocompatible composition comprises:

- a density between 997 and 1600 kg/m³;
- a resting viscosity, measured using a rheometer at 25°C, ranging from 0.01 to 1.5 Pa·s; and
- a maximum sedimentation velocity of the cellular elements with respect to
10 the solution less than $5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or $20 \text{ mm} \cdot \text{h}^{-1}$.

[0113] According to a specific embodiment, the composition according to the invention, which has a maximum sedimentation velocity of the cellular elements with respect to the solution of less than $2.78 \times 10^{-7} \text{ m} \cdot \text{s}^{-1}$, or 1
15 $\text{mm} \cdot \text{h}^{-1}$, can be stored for up to 6 hours prior to use. Thus, in this embodiment, the composition according to the invention maintains the uniform distribution of the heterogeneous cellular elements for 6 hours.

[0114] Advantageously, the composition according to the invention can be
20 stored for a long period without affecting the uniform distribution of the heterogeneous cellular elements, thereby ensuring precise dosing, particularly during injection.

[0115] The composition according to the invention comprises a solution and
25 heterogeneous cellular elements suspended in said solution. Said cellular elements being selected from isolated cells, cellular microtissues, cell clusters, and mixtures thereof, of which at least two, preferably a majority, and even more preferably all of said cellular elements have a different density and/or size and/or number of cells.

30

[0116] According to a specific embodiment of the invention, the composition comprises at least one encapsulated cellular element, preferably contained

within a three-dimensional microcompartment comprising an outer hydrogel layer. The microcompartment can be a solid hydrogel sphere. Preferably, the composition comprises at least one encapsulated cellular element, preferably contained within 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 a particular embodiment, the outer hydrogel layer of the microcompartment can comprise or be made 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, preferably greater than 60 kPa, more preferably 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 preferably 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 preferably the concentration is equal to 2% (plus or minus 0.5%) by mass.

20

[0117] 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. Preferably, the cells constituting all or part of the cellular elements are selected from neural cells, neuronal cells, glial cells, cardiac cells, retinal cells, lymphocytes, hepatocytes, endothelial cells, mesenchymal stem cells, keratinocytes, fibroblasts, pre-adipocytes, adipocytes, pancreatic islet cells, and mixtures thereof.

30 [0118] Advantageously, the composition according to the invention is suitable for keeping all cell types in suspension, whether or not the cellular elements are encapsulated.

[0119] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the cellular elements consist of at least two different cell types.

[0120] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein at least 20%, preferably at least 30%, and in particular at least 40% of the cellular elements are in the form of a microtissue.

[0121] According to one embodiment, the composition according to the invention comprises between 10 and 55% cellular elements in the form of a microtissue.

[0122] 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, preferably, a largest dimension of at least 50 μm , more preferably between 50 μm and 1.3 mm, in particular between 100 μm and 800 μm .

[0123] Thus, in this embodiment, the biocompatible composition according to the invention comprises at least one microtissue whose largest dimension is at least 50 μm . Unexpectedly, the inventors succeeded in keeping the cellular elements, comprising at least one microtissue in this embodiment, in suspension in a liquid composition. This feature makes the composition easy to inject.

[0124] 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.

[0125] Additionally, it is advantageous that the biocompatible composition not be in gel form.

- 5 [0126] 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%, preferably 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 are in the form of isolated cells.

10

[0127] According to one embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, in which no cellular element is in the form of an isolated cell.

- 15 [0128] In the context of the invention, the percentage of cellular elements in the form of microtissue or isolated cells is expressed as the cumulative volume of the cellular elements.

- [0129] According to one embodiment, the composition according to the
20 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, preferably between 0.01 and 60%, in particular between 0.01 and 50%, more preferably between 0.01 and 45%, and even more preferably between 15 and 30%.

25

[0130] 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, preferably less than 1 mm, in particular less than 700 μm , and most
30 preferably less than 500 μm .

[0131] Preferably, 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, preferably between 100 μm and 800 μm .

5

[0132] 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 than 1.3 mm, preferably less than 700 μm , and most preferably less than 500 μm .

10

[0133] According to a specific 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 mL of solution, preferably at least 5 million cells, and more particularly 50 million cells.

15

[0134] 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, preferably between 1 and 50 million cells, more particularly between 1 and 10 million cells, more preferably between 2 and 6 million cells, and even more preferably between 3 and 5 million cells per mL of solution.

20

[0135] According to another embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises:

25

- water;
- at least one biocompatible density-adjusting agent; and
- at least one biocompatible viscosity-enhancing agent, preferably at least one biocompatible viscosity-enhancing agent having a molecular weight between 0.05 MDa and 5 MDa.

30

[0136] Advantageously, the density-adjusting and viscosity-enhancing agents make it possible to adjust the rheological properties of the solution so that the density of the composition satisfies inequality [math 1].

5 [0137] Preferably, the composition according to the invention also comprises at least one biocompatible osmolality-adjusting agent. Said 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.

10 [0138] Preferably, at least one osmolality-adjusting agent for the solution is selected from mannitol, sorbitol, sodium chloride, and mixtures thereof, or modified forms of these molecules.

15 [0139] According to another embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises at least one biocompatible density-adjusting agent selected from dextran, mannitol, sodium chloride, glycerol, or sorbitol, and mixtures thereof, or modified forms of these molecules.

20 [0140] The density-adjusting agents suitable for the composition according to the invention are selected based on several parameters, namely:

- their biocompatibility, i.e., they must not affect the survival of the cellular elements;

- their high solubility in solution;

25 - their minimal effect on the viscosity of the solution.

[0141] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises at least one density-adjusting agent, preferably dextran. When the
30 solution contains dextran, the dextran preferably has a molecular weight between 20 and 80 kDa, specifically 30, 35, or 40 kDa.

[0142] According to another embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises at least one biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl cellulose (CMC), methylcellulose, hydroxyethylcellulose, ethylhydroxyethylcellulose, hydroxypropylmethylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide, polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules.

10

[0143] The viscosity-enhancing agents suitable for the composition according to the invention are selected based on several parameters, namely:

- their biocompatibility, i.e., they must not affect the survival of the cellular elements;

15 - their stability at 37°C, the temperature at which they are administered to humans.

[0144] Preferably, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises at least one viscosity-enhancing agent selected from sodium hyaluronate and/or CMC. When the solution contains sodium hyaluronate, the sodium hyaluronate preferably has a molecular weight of less than 1 MDa, more preferably between 80 and 1 MDa, and in particular between 80 and 600 kDa.

25

[0145] When the solution contains CMC, the CMC preferably has a molecular weight between 40 kDa and 1 MDa, and even more preferably between 50 and 400 kDa.

30 [0146] 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 when administered by injection.

[0147] According to another embodiment, the composition according to the invention comprises:

- between 0.01 and 30% by mass of density-adjusting agent(s) with respect to the total mass of the solution
- between 0.01 and 8% by mass of viscosity-enhancing agent(s) with respect to the total mass of the solution.

[0148] According to one particularly preferred embodiment, the composition according to the invention comprises a solution and cellular elements suspended in said solution, wherein the solution comprises:

- sodium hyaluronate and/or CMC; and
- dextran.

[0149] 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 with molecular weights of 20 kDa and 80 kDa, with respect to the total mass of the solution;
- between 0.01 and 7% by mass of CMC with a molecular weight ranging from 40 kDa to 1 MDa, with respect to the total mass of the solution, and/or between 0.0 and 7% by weight of sodium hyaluronate with molecular weights ranging from 80 kDa to 1 MDa, with respect to the total mass of the solution

[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 particularly preferred embodiment, the composition according to the invention is a biocompatible composition obtained by a preparation method comprising the following steps:

30

[0151] (a) Preparation or recovery of a composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density

and/or size and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least 50 μm ;

- [0152] (b) Preparation of a biocompatible composition comprising the cellular elements of the composition from step ((a), solution (B), and optionally solution (A), such that the difference in density between the density of the solution of the composition obtained in step (b) and the average density of the cellular elements of the composition obtained in step (b) satisfies the following inequality:

10

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

- 15 where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

20 where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

25 and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$;

- (c) Optionally, recovery of the composition obtained in step (b), comprising a solution and cellular elements suspended in said solution, at least two of which cellular elements have a different density and/or size and/or number of cells, preferably at least one cellular element being a microtissue having a largest dimension of at least 50 μm , wherein said composition is characterized in that

30

the difference in density between the density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b).

- 5 [0153] Preferably, when step (a) involves preparing a composition, it comprises carrying out the following steps:
- 1) Culturing at least one cellular element;
 - 2) Rinsing the cell culture from step 1);
 - 3) Centrifuging the cell culture obtained in step 2);
- 10 4) Recovering a composition comprising cellular elements resulting from the centrifuging of step 3), at least two of which have a different density and/or size and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least 50 μm .
- 15 [0154] According to a preferred embodiment, step (b) comprises carrying out the following steps:
- (b1) Characterizing at least one rheological parameter selected from:
- *the density of the cellular element with the lowest density and the density of the cellular element with the highest density among said cellular elements
- 20 present in the composition obtained in step (a);
- *the density of the composition obtained in step (a);
 - *the viscosity of the composition obtained in step (a);
 - *the largest dimension of the cellular elements present in the composition obtained in step (a)
- 25 (b2) Preparing a solution (B) comprising at least one density-adjusting agent and/or at least one viscosity-enhancing agent and water;
- (b3) Sterilizing solution (B) from step (b2) to obtain a sterilized solution (B);
- (b4) Optionally, adjusting the pH of the sterilized solution (B) from step (b3);
- (b5) Preparing a biocompatible composition comprising the cellular elements
- 30 of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A) such that the difference in density between the density of the solution obtained in step (b5)

and the average density of the cellular elements of the composition obtained in step (b5) satisfies the inequality mentioned in step (b).

[0155] Preferably, step (b2) for preparing solution (B) is carried out by adding
5 a biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl
cellulose (CMC), methylcellulose, hydroxyethylcellulose,
ethylhydroxyethylcellulose, hydroxypropylmethylcellulose (HPMC), alginate,
pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide,
polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium
10 hyaluronate, and mixtures thereof, or modified forms of these molecules.

[0156] Preferably, step (b2) for preparing solution (B) is carried out by adding
at least one biocompatible density-adjusting agent selected from dextran,
mannitol, NaCl, glycerol, sorbitol, and mixtures thereof, or modified forms of
15 these molecules.

[0157] Preferably, step (b2) for preparing solution (B) also comprises adding at
least one element selected from sodium chloride (NaCl), calcium chloride
(CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures
20 thereof.

[0158] According to one embodiment, step (b) comprises carrying out the
following steps:

(b1) Characterizing at least one rheological parameter selected from:

- 25 *the density of the cellular element with the lowest density and the density of
the cellular element with the highest density among said cellular elements
present in the composition obtained in step (a);
*the density of the composition obtained in step (a);
*the viscosity of the composition obtained in step (a);
30 *the largest dimension of the cellular elements present in the composition
obtained in step (a)

(b2) Preparing a solution (B) comprising at least one density-adjusting agent and/or at least one viscosity-enhancing agent and water;

(b4) Optionally, adjusting the pH of solution (B) obtained in step (b2);

5 (b3) Sterilizing solution (B) obtained in step (b4) to obtain a sterilized solution (B);

(b5) Preparing a biocompatible composition comprising the cellular elements of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A) such that the difference in density between the density of the solution obtained in step (b5)

10 and the average density of the cellular elements of the composition obtained in step (b5) satisfies the inequality mentioned in step (b).

[0159] In this embodiment, step (b4), in which the pH of solution (B) is adjusted, is carried out prior to step (b3), in which solution (B) is sterilized.

15 Method for preparing a biocompatible composition according to the invention

[0160] 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:

20 (a) Preparation or recovery of a composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density and/or size and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least 50 μm ;

25 (b) Preparation of a biocompatible composition comprising the cellular elements of the composition from step (a), solution (B), and optionally solution (A), such that the difference in density between the density of the solution of the composition obtained in step (b) and the average density of the cellular elements of the composition obtained in step (b) satisfies the following inequality:

30

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m³, and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m³,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in m·s⁻¹;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in Pa·s, where g represents the acceleration due to gravity expressed in m·s⁻²;

and where $v_c = 5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$;

(c) Optionally, recovery of the composition obtained in step (b), comprising a solution and cellular elements suspended in said solution, at least two of which cellular elements have a different density and/or size and/or number of cells, preferably at least one cellular element being a microtissue having a largest dimension of at least 50 µm, wherein said composition is characterized in that the difference in density between the density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b).

[0161] Advantageously, the method according to the invention makes it possible to achieve a balance between density and viscosity depending on the heterogeneous cellular elements, such that the maximum sedimentation velocity of the cellular elements with respect to the solution is less than $5.56 \cdot 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or $20 \text{ mm} \cdot \text{h}^{-1}$.

[0162] According to one embodiment, the composition of step (a) comprises a solution in which said cellular elements are suspended.

[0163] According to a preferred embodiment, the composition of step (a) has a maximum sedimentation velocity of the cellular elements with respect to the solution greater than $5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or $20 \text{ mm} \cdot \text{h}^{-1}$.

5

[0164] Preferably, when step (a) involves preparing a composition, it comprises carrying out the following steps:

1) Culturing at least one cellular element;

2) Rinsing the cell culture from step 1);

10 3) Centrifuging the cell culture obtained in step 2);

4) Recovering a composition comprising cellular elements resulting from the centrifuging of step 3), at least two of which have a different density and/or size and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least $50 \mu\text{m}$.

15

[0165] According to one embodiment, the cell culture described in step 1) is carried out in a bioreactor.

20

[0166] According to a specific embodiment of the invention, at least one cellular element of step 1) comprises at least one encapsulated cell, preferably encapsulated in alginate.

[0167] Preferably, at least one cellular element from step 1) is contained within a three-dimensional microcompartment comprising an outer hydrogel layer.

25

[0168] When step 1) comprises culturing at least one encapsulated cellular element, step (a) may also comprise a step 2') aimed at removing the capsule in order to recover the cellular element prior to step 3) of centrifugation.

30

[0169] Step 2') consists in removing the capsule, preferably by removing the outer hydrogel layer. Preferably, step 2') may be carried out, in particular, by hydrolysis, dissolution, perforation, and/or rupture using any biocompatible

method, i.e., one that is non-toxic to cells. For example, removal can be achieved using a phosphate-saline buffer, a divalent ion chelator, an enzyme such as alginate lyase if the hydrogel comprises alginate, and/or laser microdissection.

5

[0170] According to one embodiment, the composition of step (a) comprises at least two distinct cell types.

[0171] Preferably, step (b) comprises carrying out the following steps:

10 (b1) Characterizing at least one rheological parameter selected from:

*the density of the cellular element with the lowest density and the density of the cellular element with the highest density among said cellular elements present in the composition obtained in step (a);

*the density of the composition obtained in step (a);

15 *the viscosity of the composition obtained in step (a);

*the largest dimension of the cellular elements present in the composition obtained in step (a)

(b2) Preparing a solution (B) comprising at least one density-adjusting agent and/or at least one viscosity-enhancing agent and water;

20 (b3) Sterilizing solution (B) from step (b2) to obtain a sterilized solution (B);

(b4) Optionally, adjusting the pH of the sterilized solution (B) from step (b3);

(b5) Preparing a biocompatible composition comprising the cellular elements of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A) such that the
25 difference in density between the density of the solution obtained in step (b5) and the average density of the cellular elements of the composition obtained in step (b5) satisfies the following inequality:

[Math 1]

30

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in

5 kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

10 where $F = 18\cdot\mu_c/g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

15 [0172] Preferably, step (b2) for preparing solution (B) is carried out by adding a biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl cellulose (CMC), methylcellulose, hydroxyethylcellulose, ethylhydroxyethylcellulose, hydroxypropylmethylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide,

20 polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules.

[0173] Preferably, step (b2) for preparing solution (B) is carried out by adding at least one biocompatible density-adjusting agent selected from dextran,

25 mannitol, NaCl, glycerol, sorbitol, and mixtures thereof, or modified forms of these molecules.

[0174] Preferably, step (b2) for preparing solution (B) also comprises adding at least one element selected from sodium chloride (NaCl), calcium chloride

30 (CaCl_2) , potassium chloride (KCl), magnesium chloride (MgCl_2) , and mixtures thereof.

[0175] Advantageously, a person skilled in the art will be able to tailor the density and viscosity by varying the concentrations of at least one density-adjusting agent and/or at least one viscosity-enhancing agent.

5

[0176] According to a particular embodiment, solution (B) from step (b2) comprises:

- between 10 and 20% by mass with respect to the total mass of the dextran solution, preferably having molecular weights of 20 kDa and 80 kDa;

- 10 - between 3 and 6% by mass of CMC, preferably having a molecular weight between 40 and 1 MDa, with respect to the total mass of the solution, and/or between 0.3 and 10% by weight of sodium hyaluronate with molecular weights between 80 and 800 kDa.

- 15 [0177] According to a preferred embodiment, solution (B) from step (b2) is prepared under stirring, preferably using a paddle mixer or a magnetic stir bar.

[0178] Preferably, sterilization in step (b3) is performed by filtration through a mesh with a pore size between 0.01 and 0.22 μm .

20

[0179] According to one embodiment, step (b4) consists in adding a biocompatible pH buffer, preferably selected from phosphate buffer, citric acid, or HEPES, and more preferably the phosphate-saline buffer listed under number 4004200 in the European Pharmacopoeia, to the sterilized solution (B)

25 from step (b3) so that the sterilized solution (B) has a pH between 7 and 7.6.

30

[0180] According to one embodiment, step (b5) of preparing a composition comprising the cellular elements of the composition obtained in step (a) with the sterilized solution (B) obtained in step (b3), or (b4), comprising a step of extracting the cellular elements from the composition obtained in step (A). In other words, according to this embodiment, step (b5) comprises an extraction

step aimed at isolating the cellular elements from solution (A) present in the composition obtained in step (a).

[0181] Thus, according to one embodiment, the method for preparing a composition according to the invention comprises carrying out the following steps:

[0182] (a) Preparation or recovery of a composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density and/or size and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least 50 μm ;

[0183] (b1) Characterizing at least one rheological parameter selected from:
*the density of the cellular element with the lowest density and the density of the cellular element with the highest density among said cellular elements present in the composition obtained in step (a);

*the density of the composition obtained in step (a);

*the viscosity of the composition obtained in step (a);

*the largest dimension of the cellular elements present in the composition obtained in step (a)

(b2) Preparing a solution (B) comprising at least one density-adjusting agent and/or at least one viscosity-enhancing agent and water;

(b3) Sterilizing solution (B) from step (b2) to obtain a sterilized solution (B);

(b4) Optionally, adjusting the pH of sterilized solution (B) obtained in step (b3);

(b5) Preparing a biocompatible composition comprising the cellular elements of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A) such that the difference in density between the density of the solution obtained in step (b5) and the average density of the cellular elements of the composition obtained in step (b5) satisfies the following inequality:

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

5 where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m³, and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m³,

where D represents the largest dimension of the cellular elements, expressed in meters,

10 where v_c represents the acceptable sedimentation velocity threshold expressed in m·s⁻¹;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in Pa·s, where g represents the acceleration due to gravity expressed in m·s⁻²;

15 and where $v_c := 5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$

(c) Optionally, recovery of the biocompatible composition obtained in step (b), comprising a solution and cellular elements suspended in said solution, at least two of which cellular elements have a different density and/or size and/or number of cells, wherein said composition is characterized in that the
20 difference in density between the density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b5).

[0184] According to another embodiment, the method for preparing a
25 composition according to the invention comprises the following steps:

(a) Preparation or recovery of a composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density and/or size and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least 50 µm comprising the
30 following steps:

1) Culturing at least one cellular element;

- 2) Rinsing the cell culture from step 1);
- 3) Centrifuging the cell culture obtained in step 2);
- 4) Recovering a composition comprising cellular elements resulting from the centrifuging of step 3), at least two of which have a different density and/or size
- 5 and/or number of cells, preferably, at least one cellular element is a microtissue having a largest dimension of at least 50 μm ;
- (b1) Characterizing at least one rheological parameter selected from:
- *the density of the cellular element with the lowest density and the density of the cellular element with the highest density among said cellular elements
- 10 present in the composition obtained in step (a);
- *the density of the composition obtained in step (a);
- *the viscosity of the composition obtained in step (a);
- *the largest dimension of the cellular elements present in the composition obtained in step (a)
- 15 (b2) Preparing a solution (B) comprising at least one density-adjusting agent and/or at least one viscosity-enhancing agent and water;
- (b3) Sterilizing solution (B) from step (b2) to obtain a sterilized solution (B);
- (b4) Optionally, adjusting the pH of the sterilized solution (B) from step (b3);
- (b5) Preparing a biocompatible composition comprising the cellular elements
- 20 of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A) such that the difference in density between the density of the solution obtained in step (b5) and the average density of the cellular elements of the composition obtained in step (b5) satisfies the following inequality:

25

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

- 30 where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular

elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

- 5 where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18.\mu_c/g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

- 10 and where $v_c := 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$

- (c) Optionally, recovery of the biocompatible composition obtained in step (b), comprising a solution and cellular elements suspended in said solution, at least two of which cellular elements have a different density and/or size and/or number of cells, wherein said composition is characterized in that the
15 difference in density between the density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b5).

- [0185] According to one embodiment, the maximum sedimentation velocity of
20 the cellular elements from step (a) in solution (A) is greater than $5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$, or $20 \text{ mm}\cdot\text{h}^{-1}$.

- [0186] According to one embodiment, the method according to the invention comprises a step of cryopreserving the composition obtained in step (a). When
25 the method according to the invention comprises a step of cryopreserving the composition obtained in step (a), said method also comprises a step of thawing the cryopreserved composition.

- [0187] According to one embodiment, step (b) comprises carrying out the
30 following steps:

(b1) Characterizing at least one rheological parameter selected from:

*the density of the cellular element with the lowest density and the density of the cellular element with the highest density among said cellular elements present in the composition obtained in step (a);

*the density of the composition obtained in step (a);

5 *the viscosity of the composition obtained in step (a);

*the largest dimension of the cellular elements present in the composition obtained in step (a)

(b2) Preparing a solution (B) comprising at least one density-adjusting agent and/or at least one viscosity-enhancing agent and water;

10 (b4) Optionally, adjusting the pH of solution (B) obtained in step (b2);

(b3) Sterilizing solution (B) from step (b4) to obtain a sterilized solution (B);

(b5) Preparing a biocompatible composition comprising the cellular elements of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A) such that the

15 difference in density between the density of the solution obtained in step (b5) and the average density of the cellular elements of the composition obtained in step (b5) satisfies the inequality mentioned in step (b).

[0188] In this embodiment, step (b4), in which the pH of solution (B) is adjusted,
20 is carried out prior to step (b3), in which solution (B) is sterilized.

Composition according to the invention for use thereof

[0189] According to another aspect, the invention relates to the composition
25 according to the invention for use as a medicament.

[0190] Advantageously, the biocompatible composition according to the invention exhibits rheological characteristics and a maximum sedimentation velocity of the cellular elements that are particularly well-suited for use in cell
30 therapy.

[0191] Preferably, the composition according to the invention can be used in the treatment of diseases through cell therapy.

5 [0192] The relative velocity of the cellular elements advantageously makes it possible to control the number of cellular elements in a given volume of the composition.

10 [0193] According to one embodiment, the composition according to the invention can be used for multiple purposes. In other words, the composition can be used to carry out multiple injections while maintaining the viability of the cellular elements, even if the injections are spaced several hours apart.

15 [0194] Advantageously, the maximum sedimentation velocity of the cellular elements makes it possible to inject, for a constant volume, a quantity of cellular elements with a low standard deviation—preferably a standard deviation of less than 20%.

20 [0195] Preferably, the composition according to the invention can be used as an injectable composition.

[0196] According to one embodiment, the composition according to the invention may be administered by injection using an injection means at the injection site.

25 [0197] Preferably, the injection means is a cannula.

30 [0198] Advantageously, controlling the rheological properties of the composition according to the invention makes it possible to optimize the delivery of cellular elements and, as a result, therapeutic cells, in a precise and effective manner. Controlling these parameters makes it possible to fine-tune the injection flow rate and injection volume, thereby ensuring a uniform distribution of cellular elements in the target area.

Kit 1

[0199] According to another aspect, the invention relates to a kit comprising:

- 5 - a biocompatible composition according to any one of the embodiments described above; and
 - an injection means, preferably a cannula.

10 [0200] Advantageously, the kit according to the invention provides the practitioner with all the necessary elements to optimize the effectiveness of the injection of the cellular elements.

[0201] According to one embodiment, the kit according to the invention comprises:

- 15 - a cannula
 - a biocompatible composition according to any one of the embodiments described above, in which the cellular elements have a largest dimension less than 0.8 times the smallest internal diameter of the cannula, preferably less than 0.75, specifically between 0.2 and 0.7.

20

[0202] According to a specific embodiment of the invention, the kit according to the invention comprises a biocompatible composition in solid form, as described in any one of the embodiments described above.

25 [0203] Preferably, the kit according to the invention also comprises water.

[0204] According to one embodiment, the kit according to the invention also comprises an element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

30

Kit 2

[0205] Finally, according to a final aspect, the invention relates to a kit comprising:

- a biocompatible preparation (1) comprising at least one biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl cellulose (CMC), methyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, hydroxypropyl methylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide, polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules, and at least one biocompatible density-adjusting agent selected from dextran, mannitol, NaCl, glycerol, sorbitol, and mixtures thereof, or modified forms of these molecules.
- a biocompatible preparation (2) 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 number of cells, preferably at least one cellular element is a microtissue having a largest dimension of at least 50 μm so that the mixture of preparation (1) and preparation (2) forms a composition in which the difference in density between the density of preparation (1) and the average density of the cellular elements of preparation (2) satisfies the following inequality:

[Math 1]

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

- 5 where $F = 18.\mu_c/g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;
and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

- 10 [0206] According to a preferred embodiment, the biocompatible preparation (2) comprises a solution.

[0207] Preferably, the kit according to the invention also comprises water.

- 15 [0208] According to one embodiment, the kit according to the invention comprises a biocompatible preparation (1) in solid form.

[0209] According to one embodiment, the preparation (1), in solid form, comprising at least one biocompatible viscosity-enhancing agent selected from

- 20 PVA, carboxymethyl cellulose (CMC), methyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, hydroxypropyl methylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide, polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules, and
25 at least one biocompatible density-adjusting agent selected from dextran, mannitol, NaCl, glycerol, sorbitol, and mixtures thereof, or modified forms of these molecules.

- [0210] According to one embodiment, the kit according to the invention also
30 comprises an element selected from sodium chloride (NaCl), calcium chloride

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

Examples

5

Example 1

[0211] The purpose of this example is to evaluate the sedimentation velocity of cellular elements in different compositions, namely a composition comprising cellular elements and an equilibrated Hank's saline solution (A) (HBSS) (outside the scope of the invention), and the composition according to the invention comprising a solution (B) developed to maintain the uniform distribution of cellular elements and prevent clogging of the injection system.

15 [0212] 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.

[0213] In this example, the cellular elements consist of a mixture of microtissues with a largest dimension of at least 50 µm, isolated cells, and aggregates of neuronal cells.

[0214] 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: 1056 kg/m³;

Largest dimension of the cellular elements: 200 µm, or 0.0002 m.

[0215] Solution (B), described in Table 1 below, was prepared under sterile conditions and characterized in Table 2.

[Table 1]

Reagent	Reference	Test concentration
HA100	PHI4468P02E (V.I. : 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 H O687	CAS 77-92-9	0.25 mg/mL
Na ₂ HPO ₄	CAS 7558-79-4	2.50 mg/mL

[Table 2]

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

5

Solution (A) was also prepared under sterile conditions and characterized in Table 3.

[Table 3]

10

Physicochemical parameters of the solution (B)	Value
Resting viscosity	0.0012 Pa·s
Density	1002 kg/m ³

Preparation of the compositions

Phase 1: Prepare the cellular elements

15

[0216] 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 at 37°C and removed when only a small piece of ice remained (≈2 min).

20

[0217] 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.

- 5 [0218] The vials were rinsed with 1 mL of HBSS (calcium, magnesium, glucose) to collect as many cellular elements as possible

[0219] The 25 mL tube was centrifuged at 300 G for 5 minutes, and most of the supernatant was removed using a P1000 pipette followed by a P20 pipette.

10

[0220] The cellular elements were resuspended in 1 mL of HBSS (calcium, magnesium, glucose).

Phase 2: Divide the samples into aliquots

15

[0221] An unspecified volume and concentration of the cell suspension were aliquoted into two 1.5 mL Eppendorf tubes.

[0222] The 1.5 mL tubes were centrifuged at 300 G for 5 minutes, and most of the supernatant was removed using a P1000 filter followed by a P20 filter.

20

[0223] 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).

- 25 Phase 3: Assess the sedimentation velocity

[0224] The tubes were installed on the assembled device so that photos could be taken.

- 30 [0225] Photos were taken every minute for 12 hours; see Figure 1.

[0226] Using ImageJ software, the trajectories of the cellular elements were manually tracked on a time-lapse video recorded with the camera in order to evaluate the sedimentation velocity.

- 5 [0227] An average of 5 sedimentation velocities for cellular elements in solution (B), over 10 of 12 trajectories, was calculated to estimate the maximum sedimentation velocity. In Solution (A), only three velocities of cellular elements, over the course of four trajectories, were used to determine the value, due to rapid sedimentation and the difficulty of tracking the exact
10 same cellular element.

[0228] The calculated maximum sedimentation velocity (the average of the 5 maximum velocities for solution (B) and the average of the 3 maximum velocities for solution (A) of microtissues) is:

- 15 * about 363 mm/h in solution (A), outside the scope of the invention;
* about 1.66 mm/h in solution (B) according to the invention, in which the difference in density between the solution and the average density of the cellular elements of the composition obtained in step (b5) satisfies inequality [Math 1].

20

[0229] Table 4 below verifies inequality [Math 1] for solution (A) and solution (B) with $V_c = 5.56 \times 10^{-6}$ m/s.

[Table 4]

25

	$ \Delta\rho = \rho_{MT} - \rho_s$	$F \cdot v_c / D^2$	$ \Delta\rho < F \cdot v_c / D^2$
Solution (A), outside the scope of the invention	54	0.03	NO
Solution (B) Invention	7	11.6	YES

Conclusions

[0230] The composition according to the invention, comprising the cellular elements and solution (B), allows said cellular elements to remain in suspension in said solution (B) for several hours. In the composition according to the invention, certain cellular elements are still in suspension after 12 hours of sedimentation.

[0231] However, the composition outside the scope of the invention comprises the cellular elements, and solution (A) does not allow the cellular elements to be maintained in suspension in said solution (A) over the long term. The cellular elements settle very quickly in low-viscosity solutions with no density difference, such as HBSS (calcium, magnesium, glucose), which has a resting viscosity of about 1 mPa·s and a density of about 1.0038 g/cm³

Example 2

[0232] The purpose of this example is to evaluate the viability of the cellular elements after 12 hours of sedimentation within the composition according to the invention.

[0233] To carry out this example, the composition according to the invention comprises the following elements:

[Table 5]

Reagent	Reference	Test concentration
HA100	PHI4468P02E (V.I. : 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%
C6H8 O7	CAS 77-92-9	0.025%
Na ₂ HPO ₄	CAS 7558-79-4	0.25%

[0234] The composition was also prepared under sterile conditions and characterized in Table 6.

[Table 6]

5

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

[0235] The results of the cellular element characterization made it possible to measure the density and the largest dimension of the cellular elements, namely:

10

[0236] Density of the cellular elements: 1065 kg/m³;

[0237] Largest dimension of the cellular elements: 200 μm , or 0.0002 m;

15 [0238] 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.

Experimental Protocol

20

Conditions

[0239] Production batches are identified by the prefix "PAR," followed by an incremental code ranging from 19 to 22. They correspond to successive
25 production batches of cellular elements.

[0240] 1) For each batch, two types of samples are prepared:

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

- 5 [0241] Composition according to the invention: A suspension of cellular elements resuspended in a solution, with a ratio of 20% by volume of cellular elements to 80% by volume of solution.

[0242] 2) Once the samples have been prepared, they are stored in sealed
10 tubes at room temperature for 12 hours.

[0243] 3) The cellular elements are then rinsed in an excess of reference culture medium.

15 [0244] 4) The cellular elements are then recultured for 24 hours in a reference culture medium.

[0245] 5) Viability is measured. The result is expressed by normalizing the viability value under the immersion condition in the solution by the viability
20 obtained under the control condition.

Results

[0246] The results of this example are described in Figure 2. This experiment
25 shows an average decrease in viability of about 25% after a 12-hour immersion in the solution in question, compared to a 12-hour control in a reference culture medium. In this example, this decrease in viability is acceptable given the acceptance criterion of 70% relative viability.

30 [0247] Example 3: Evaluation of the impact of the injection method for the composition according to the invention on the viability of cellular elements.

[0248] The purpose of this example is to evaluate the impact of the method for injecting the composition according to the invention on the viability of the cellular elements.

5 [0249] In the context of the invention, it is important to ensure that the viscosity of the solution—which is necessary to maintain the uniform distribution of the cellular elements—does not induce excessive shear stress during injection, which could compromise the viability of the cells.

10 [0250] 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.

15

[0251] Because shear forces experienced during injection may have a delayed effect on cell viability, the viability of cellular elements that have undergone the injection procedure is measured immediately after injection and again after 24 hours of reculture in a reference culture medium.

20

[0252] The composition according to the invention used in this example has the same composition (Table 5) and the same rheological properties (Table 6) as the composition according to the invention disclosed in Example 2.

25 [0253] 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;

c) Injecting the composition according to the invention using the syringe from

30 step b) into a cannula at an injection 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;
- f) Placing the culture medium containing the cellular elements in an incubator for 24 hours.

5

[0254] 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.

Results

10

[0255] The viability measurements shown in Figure 3 do not indicate a significant impact of the injection procedure on viability under the conditions of the example. The slight decrease observed immediately after the injection may reflect a temporary decline in cellular metabolic activity; however, this decrease is not observed after 24 hours of culture.

15

[0256] As a result, the composition according to the invention can be used as an injectable composition.

Reagent	% w/w	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

20

Example 3: Characterization of the viscoelastic properties of a composition according to the invention using oscillatory sweep rheological measurement

[0257] To carry out this example, the composition according to the invention comprises a solution characterized in Table 7, as well as cellular elements.

25

[Table 7]

[0258] The cellular elements of the composition used in this example are a mixture of microtissues with a largest dimension of at least 50 μm , isolated
5 cells, and aggregates of neuronal cells.

[0259] Finally, certain physicochemical parameters of the composition according to the invention are characterized in Table 8 below.

10 [Table 8]

Physicochemical parameters of the composition	Value
Resting viscosity	900 $\text{mPa}\cdot\text{s}$
Density	1071 kg/m^3
pH	7.2

[0260] The behavior of the composition according to the invention was verified by standard rheological measurements using oscillatory sweep analysis with
15 an Anton Paar MCR 302 rheometer. The angular frequency is constant and set at 10 rad/s . The shear strain varies from 0.001 to 10%, and the temperature is constant and maintained at 20°C.

[0261] Two parameters were measured to characterize the viscoelastic
20 properties of the composition: the storage modulus (G') and the loss modulus (G''):

[0262] -The storage modulus (G') represents the elastic portion of the strain. It is related to the material's ability to store energy during strain and is
25 proportional to the material's stiffness. It therefore determines the magnitude of its elastic response and is consequently associated with the behavior of an elastic solid—that is, a gel.

[0263] -The loss modulus (G'') represents the viscous component of the strain. It is related to the dissipation of energy in the form of heat and is proportional to the material's damping behavior. It determines the magnitude of its viscous response and, as a result, its fluid behavior.

5

[0264] In the case of a rigid or structured material, the storage modulus (G') will be greater than the loss modulus (G''). Conversely, in the case of a liquid material, the loss modulus (G'') will be greater than the storage modulus (G').

10 [0265] The results presented in Figure 4 showed 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 4: Study of the viability of cellular elements in a solution comprising

15 CMC

[0266] The purpose of this example is to assess the viability of the cellular elements after 12 hours of immersion in a solution comprising CMC.

20 [0267] The various solutions used in this example are disclosed in Table 9 below.

[Table 9]

	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		

25

[0268] To carry out this example, the M4 solution 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.

[0269] In this example, the cellular elements consist of a mixture of microtissues with a largest dimension of at least 50 μm , isolated cells, and aggregates of neuronal cells.

- 5 [0270] The composition according to the invention was kept at room temperature for 12 hours.

[0271] The cellular elements are then rinsed in an excess of reference culture medium. The microtissues are then collected, and viability is measured using
10 a cell viability bioluminometer. The result is expressed as luminescence signals normalized by the CM control signal. To assess cell viability, 7 analytical replicates were performed for each condition.

[0272] The results of this example show that the composition according to the
15 invention comprising the M4 solution and the cellular elements remains viable (96% compared to a 12-hour control in a reference culture medium).

[0273] In conclusion, Example 4 demonstrates that the composition according
to the invention, comprising the M4 solution and cellular elements, maintains
20 high cell viability after 12 hours of immersion at room temperature.

CLAIMS

[Claim 1] A biocompatible composition, comprising a solution and cellular elements suspended in said solution, which cellular elements are 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 number of cells, said composition being characterized in that the difference in density between the density of the solution and the average density of the cellular elements satisfies the following inequality:

$$|\Delta\rho| < F \cdot v_c / D^2$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

[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 $50 \mu\text{m}$.

[Claim 3] The composition according to one of the preceding claims, characterized in that the osmolality of the cellular elements in the solution is between 180 and 360 mOsm $\cdot$ kg⁻¹.

- 5 [Claim 4] The composition according to one of the preceding claims, characterized by a density of between 800 and 2000 kg/m³.

[Claim 5] The composition according to one of the preceding claims, characterized by a resting viscosity measured at 25°C of between 0.01 and
10 1.5 Pa $\cdot$ s.

[Claim 6] The composition according to one of the preceding claims, characterized in that the solution comprises at least one ion selected from sodium ions, calcium ions, chlorine ions, potassium ions and mixtures thereof.
15

[Claim 7] The composition according to one of the preceding claims, characterized in that the solution comprises at least one element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.
20

[Claim 8] The composition according to one of the preceding claims, characterized in that it comprises:

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

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

[Claim 10] The composition according to one of the preceding claims, characterized in that the cells constituting the cellular elements are mature cells and/or progenitor cells and/or stem cells.

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[Claim 11] The 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, cardiac cells, retinal cells, lymphocytes, hepatocytes, endothelial cells, mesenchymal stem cells, keratinocytes, fibroblasts, pre-adipocytes, adipocytes, pancreatic islet cells, and mixtures thereof.

10

[Claim 12] The composition according to one of the preceding claims, characterized in that the volume fraction of the cellular elements is between 0.01% and 60% by volume.

15

[Claim 13] The composition according to one of the preceding claims, characterized in that the solution comprises:

20

- water,
- at least one biocompatible density-adjusting agent
- at least one biocompatible viscosity-enhancing agent, preferably having a molecular weight between 0.05 MDa and 5 MDa.

[Claim 14] The composition according to the preceding claim, characterized in that the solution also comprises at least one biocompatible osmolality-adjusting agent.

25

[Claim 15] The composition according to the preceding claim, characterized in that the at least one density-adjusting agent is selected from dextran, mannitol, NaCl, glycerol or sorbitol and mixtures thereof, or modified forms of these molecules.

30

[Claim 16] The composition according to one of claims 13 to 15, characterized in that the biocompatible viscosity-enhancing agent is selected from PVA, carboxymethyl cellulose (CMC), methylcellulose, hydroxyethylcellulose, ethylhydroxyethylcellulose, hydroxypropylmethylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide, polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules.

10 [Claim 17] The composition according to one of claims 13 to 16, characterized in that it comprises:

- between 0.01 and 30% by mass of density-adjusting agent(s) with respect to the total mass of the solution
- between 0.01 and 8% by mass of viscosity-enhancing agent with respect to the total mass of the solution.

15

[Claim 18] The composition according to one of the preceding claims, characterized in that the composition is obtained by carrying out the following steps:

20 (a) Preparation or recovery of a biocompatible composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density and/or size and/or number of cells;

(b) Preparing a biocompatiblebiocompatible composition comprising the cellular elements of the composition obtained in step (a) and a solution (B), and optionally solution (A), such that the difference in density between the density of the solution and the average density of the cellular elements of the composition obtained in step (b) satisfies the following inequality:

25

$$|\Delta\rho| < F \cdot v_c / D^2$$

30

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of

the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

5 where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18.\mu_c/g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

10 and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

(c) Optionally, recovery of the biocompatible composition obtained in step (b), comprising a solution and cellular elements suspended in said solution, at least two of which cellular elements have a different density and/or size and/or number of cells, wherein said difference in density between the
15 density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b).

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

20

[Claim 20] The composition for use according to the preceding claim, to be injected using a cannula at the injection site.

[Claim 21] A method for preparing a composition according to one of the
25 preceding claims, characterized in that it comprises carrying out the following steps:

(a) Preparation or recovery of a composition comprising a solution (A) and a set of cellular elements, at least two of which have a different density and/or size and/or number of cells;

30 (b) Preparing a biocompatible composition comprising the cellular elements of the composition obtained in step (a) and a solution (B), and optionally solution (A), such that the difference in density between the density

of the solution and the average density of the cellular elements of the composition obtained in step (b) satisfies the following inequality:

$$|\Delta\rho| < F \cdot v_c / D^2$$

5

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

10 where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

15 where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

and where $v_c = 5.56 \times 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

(c) Optionally, recovery of the biocompatible composition obtained in step (b), comprising a solution and cellular elements suspended in said
20 solution, at least two of which cellular elements have a different density and/or size and/or number of cells, wherein said difference in density between the density of the solution and the average density of the cellular elements satisfies the aforementioned inequality in step (b).

25 [Claim 22] The method according to the preceding claim, characterized in that step (a) comprises carrying out the following steps:

- 1) Culturing at least one cellular element;
- 2) Rinsing the cell culture from step 1);
- 3) Centrifuging the cell culture obtained in step 2);

4) Recovering a composition comprising cellular elements resulting from the centrifuging of step 3), at least two of which have a different density and/or size and/or number of cells.

5 [Claim 23] The method according to the preceding claim, characterized in that at least one cellular element comprises at least one encapsulated cell, preferably encapsulated in alginate.

[Claim 24] The method according to one of claims 21 to 23, characterized in
10 that the composition of step (a) has a maximum sedimentation velocity of the cellular elements with respect to the solution greater than $5.56 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$, or $20 \text{ mm} \cdot \text{h}^{-1}$.

[Claim 25] The method according to one of claims 21 to 24, characterized in
15 that step (b) comprises carrying out the following steps:

(b1) Characterizing at least one rheological parameter selected from:

*the density of the cellular element with the lowest density and the density of the cellular element with the highest density among said cellular elements present in the composition obtained in step (a);

20 *the density of the composition obtained in step (a);

*the viscosity of the composition obtained in step (a);

*the largest dimension of the cellular elements present in the composition obtained in step (a)

(b2) Preparing a solution (B) comprising at least one density-adjusting
25 agent and/or at least one viscosity-enhancing agent and water;

(b3) Sterilizing solution (B) from step (b2) to obtain a sterilized solution (B);

(b4) Optionally, adjusting the pH of sterilized solution (B) obtained in step (b3);

30 (b5) Preparing a composition comprising the cellular elements of the composition obtained in step (a) together with the sterilized solution (B) obtained in step (b3) or (b4) and, optionally, with solution (A), such that the

difference in density between the density of the solution obtained in step (b5) and the average density of the cellular elements of the composition obtained in step (b5) satisfies the following inequality:

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

Where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

and where $v_c = 5.56 \cdot 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

[Claim 26] The method according to the preceding claim, characterized in that step (b2) for preparing a solution (B) is carried out by adding at least one biocompatible density-adjusting agent selected from dextran, mannitol, NaCl, glycerol, sorbitol, and mixtures thereof, or modified forms of these molecules.

[Claim 27] The method according to one of claims 25 or 26, characterized in that step (b2) for preparing a solution (B) is carried out by adding a biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl cellulose (CMC), methylcellulose, hydroxyethylcellulose, ethylhydroxyethylcellulose, hydroxypropylmethylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide,

polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules.

5 [Claim 28] The preparation method according to one of claims 25 to 27, characterized in that step (b2) for preparing a solution (B) also comprises adding at least one element selected from sodium chloride (NaCl), calcium chloride (CaCl₂), potassium chloride (KCl), magnesium chloride (MgCl₂), and mixtures thereof.

10 [Claim 29] The preparation method according to one of claims 25 to 28, characterized in that step (b2) is carried out under stirring.

[Claim 30] A kit comprising:

- a biocompatible composition according to any one of claims 1 to 20;
- 15 and
- an injection means, preferably a cannula.

[Claim 31] The kit according to the preceding claim, characterized in that the biocompatible composition is in solid form.

20

[Claim 32] A kit comprising:

- A biocompatible preparation (1) comprising at least one biocompatible viscosity-enhancing agent selected from PVA, carboxymethyl cellulose (CMC), methyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, 25 hydroxypropyl methylcellulose (HPMC), alginate, pullulan, chitosan, xanthan gum, gellan gum, polyacrylamide, polyvinylpyrrolidone, polyethylene oxide, casein, collagen, sodium hyaluronate, and mixtures thereof, or modified forms of these molecules, and at least one biocompatible density-adjusting agent selected from dextran, mannitol, NaCl, glycerol, sorbitol, and mixtures thereof, 30 or modified forms of these molecules;
- A biocompatible preparation (2) 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 number of cells;

characterized in that the mixture of preparation (1) and preparation (2) forms a composition in which the difference in density between the density of preparation (1) and the average density of the cellular elements of preparation (2) satisfies the following inequality:

$$|\Delta\rho| < F \cdot \frac{v_c}{D^2}$$

where $|\Delta\rho| = \rho_s - \rho_o$ with ρ_s representing the density of the composition expressed in kg/m^3 , and ρ_o representing the average density of the cellular elements, measured using the density gradient technique and expressed in kg/m^3 ,

where D represents the largest dimension of the cellular elements, expressed in meters,

where v_c represents the acceptable sedimentation velocity threshold expressed in $\text{m}\cdot\text{s}^{-1}$;

where $F = 18 \cdot \mu_c / g \sim 9.81$, with μ_c representing the maximum static viscosity of the solution expressed in $\text{Pa}\cdot\text{s}$, where g represents the acceleration due to gravity expressed in $\text{m}\cdot\text{s}^{-2}$;

and where $v_c = 5.56 \cdot 10^{-6} \text{ m}\cdot\text{s}^{-1}$.

[Claim 33] The kit according to the preceding claim, characterized in that the preparation (2) comprises a solution.

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

[Claim 35] The kit according to one of claims 30 to 34, 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.