

COMPOSITION CONTAINING GLYCOSAMINOGLYCANS AND PROTEINS

5 **Field of invention**

The invention generally relates to compositions containing Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, chemically modified or not, and proteins, partially hydrolysed proteins or peptides, chemically modified or not, such as for example collagens.

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State of the art

The formulation of homogenous compositions containing highly hydrophilic Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, chemically modified or not, and proteins, partially hydrolysed proteins or peptides, chemically modified or not provides viscoelastic solids or semi-solids with limited mechanical properties and limited properties for biological, pharmacological or medicinal usages.

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General description of the invention

20 The problems mentioned in the previous chapter may be solved with the composition according to the present disclosure.

To this effect, the composition according to the disclosure comprise a polyhydroxyurethane, a glycosaminoglycan, a partially hydrolysed Glycosaminoglycans or Glycosamines, chemically modified or not, and proteins, partially hydrolysed proteins or peptides, chemically modified or not. The polyhydroxyurethane is obtained by the covalent coupling of cyclic carbonates (CC) and polyamines (PA) chemical functions.

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Preferably the composition is obtained by the polymerisation or covalent bound formation of a preparation containing molecules with cyclic polycarbonates functional groups and Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, or chemically modified or not with a preparation containing

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molecules with polyamines functional groups, proteins, partially hydrolysed proteins or peptides, chemically modified or not. The Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines and the proteins, partially hydrolysed or not, or the peptides may be chemically modified with the constituents of the polyhydroxyurethanes.

The Glycosaminoglycans partially hydrolysed Glycosaminoglycans or Glycosamines chemically modified with monomers or pre-polymers of the polyhydroxyurethanes can be covalently bound to proteins, partially hydrolysed proteins or peptides, chemically modified or not with monomers or pre-polymers of the polyhydroxyurethanes to give new compositions with innovative physical, biological, pharmacological or medicinal properties.

In one aspect, the present invention provides a composition comprising a first, second and third constituents, wherein said first constituent is a glycosaminoglycan, a hydrolysed glycosaminoglycan, a glycosamine, chemically modified or not or a blend of different glycosaminoglycans, hydrolysed glycosaminoglycans, glycosamines, chemically modified or not; said second constituent is a protein, a peptide or a blend of different proteins or peptides; said third constituent is a polyhydroxyurethane (PHU) which is obtained by the covalent coupling of bis or poly cyclic carbonates (CC) and di or polyamines (PA) molecules.

The composition according to the disclosure may be used in several applications such as the preparation of micro-gel or micro-particles to deliver active pharmaceutical substances or to support living cells, or for the treatment of surfaces in contact with living materials, or for the treatment of surfaces to provide them specific affinities to polysaccharides, proteins, partially hydrolysed proteins and peptides, or to chemically modify proteins, partially hydrolysed proteins and peptides to provide them novel physical, biological, pharmacological or medicinal properties or the reconstruction and the substitution of extra cellular matrix in vitro or in vivo.

The extra cellular matrix is the non cellular part presents in all tissues and organs. Its functions are essentially linked to its structure and its physical properties, but also to its biochemical composition and its essential constituents and their biomechanical functions.

In another aspect, the present invention provides a composition a for the substitution or the reconstruction of connective, epithelial, nervous, muscular, bones, cartilaginous, or dermal, animal or human extra cellular matrix for which the ratio of the number of non polymerised, or covalently bound carbonate cyclic functions from CC monomers, PRE-CC and chemically modified first and second constituents, and the number of non polymerised, or covalently bound primary and secondary amine functions from PA monomers, the PRE-PA and the chemically modified first and second constituents are between 2 and 0,5.

Fundamentally the Extra cellular Matrix (ECM) is constituted of water, proteins and polysaccharides. Every ECM processes a unique biochemical composition having in common the essential presence of proteins exhibiting various conformations such as the fibrillar structure of collagens, and glycosaminoglycans forming hydrated gels. Collagens and the other fibrillar proteins are essentially hydrophobic in the haemostatics' conditions of the ECM, while glycosaminoglycans are strongly hydrophilic. The reconstruction in vitro and in vivo of an ECM, based on those two essential families of substances, with the appropriate mechanical characteristics in biocompatible conditions is one of the applications of this innovative formulation.

When the ECM is damaged or destroyed due to different affections or traumas, or when it has been deformed, or has partially or totally disappeared due to processes associated with aging, traumas, or erosion, techniques currently exist to reconstruct or replace it.

- 5 Those techniques can be classified in three general families:
- The use of implants and biocompatible prostheses with no relation to the original composition of the ECM and without living cells.
 - The use of implants and biocompatible mechanical scaffolds based on compositions with limited relation to the original composition of the ECM, but allowing living cells
 - 10 to proliferate in the mimetic ECM.
 - Autologous grafts based on the extraction of healthy and functional ECM of the patient to reconstruct the damaged parts.

Those techniques are widely used but they all carry their well known own drawbacks, such as:

- 15 - The heavy operation traumas and the difficulties to replace prostheses without living cells.
- The weak integration and incorporation of functionalised scaffolds with neighbouring ECM.
 - The limitation to find adequate healthy ECM in autologous grafts.

- 20 The present disclosure allows reconstructing a biocompatible ECM constituted of polyhydroxyurethanes and the essential constituents of the original ECM, the proteins and the glycosaminoglycans. This ECM is formed in vitro or in vivo by the polymerisation of the monomers or pre-polymers functional groups of the polyhydroxyurethanes supporting the solubilisation and the creation of close contacts
- 25 or covalent bonds between proteins, partially hydrolysed proteins or peptides, chemically modified or not and Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines chemically modified or not in biocompatible conditions.

- 30 In some examples the composition according to the disclosure is obtained via two preparations, named "preparation a" and "preparation b" in the present document.

Carbide Corporation in the patent 3'072'613 dated 8 January 1963. The pre-polymer of the polyhydroxyurethanes of the preparation a are formulated and prepared by ways ensuring that their terminal endings are cyclic carbonate functions.

5 The chemically modified glycosaminoglycans with components of the polyhydroxyurethane are obtained following methods described in the literature and the prior art. For example, a modified Hyaluronic acid of the "GAG blend" is obtained by its deacetylation in caustic media, followed by its esterification with the cyclic polycarbonate of Diglycerol, followed by its carbamatation with the same or another
10 cyclic polycarbonate after pH adjustment between 8 and 5. The glycosaminoglycan chemically modified with any one of the polycarbonates described above can be further modified with a diamine or polyamine described in the present disclosure. The reaction of carbamatation of the side chain of the modified Hyaluronic acid is carried in the presence of an acceptable solvent, such as for example, but not limited
15 to water at a temperature between zero and 150 ° Celsius, preferably between 15 and 80° Celsius. The resulting chemically modified Hyaluronic acid can be further carbamated with a cyclic polycarbonate in the condition described above. The successive carbamatation of the modified Hyaluronic acid can be further made to construct a Polyhydroxyurethane side chain on the modified Hyaluronic acid of 3 to
20 25 monomer entities terminated with cyclic carbonates. The same procedure can be applied to partially hydrolysed Glycosaminoglycans or Glycosamines described in the disclosure.

Additionally the preparation a can contain proteins, partially hydrolysed proteins or
25 peptides, the "Blend of Proteins", chemically modified with pre-polymers of polyhydroxyurethanes or polyhydroxyurethanes, terminated with cyclic carbonates functions.

The chemically modified "Blend of Proteins" is obtained, for example by dissolving collagen in Bis(1,4-aminopropyl) Piperazine, followed by its amidation with a mixture
30 of Carbodiimide (EDC) and N-Hydroxysuccinimide, followed by the precipitation of the modified collagen in TBME (Ter-ButylMethyl Ether), followed by the dissolution and neutralisation of the wet precipitate in a solution of anhydrous Ethanol with 4% of

acetic acid, followed by a precipitation of the modified collagen in Ethyl acetate, centrifugation and drying under vacuum. Similarly to the chemical modification of the "GAG Blend" described above, this chemically modified "Blend of Proteins" can, after its amidation, be further carbamated with one of the cyclic polycarbonate described in the present disclosure in the presence of an acceptable solvent at a temperature between zero and 150° Celsius, preferably between 15 and 80° Celsius. The resulting chemically modified protein can be further carbamated with one of the diamine or polyamine described in the present disclosure. The successive carbamation of the "Blend of Proteins" can be further made to construct a Polyhydroxyurethane side chain of 2 to 26 monomers entities terminated by cyclic carbonates functions on the "Blend of Proteins".

The preparation a contains the constituents of the disclosure carrying terminal non reacted cyclic carbonates. The quantity of non reacted cyclic carbonates functions from all constituents of the preparation a is sufficient to polymerise or covalently bind to the non reacted primary or secondary amine functions from all constituents of the preparation b to obtain the desired properties from the composition containing polyhydroxyurethanes and glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, chemically modified or not and proteins, partially hydrolysed proteins or peptides, chemically modified or not.

Preparation b

The essential constituents for the intended use in the preparation b are the proteins, such as, the collagens, the elastins, the keratins, the fibronectins, the actins, the myosins, the laminins, their hydrolysed fractions or peptides; the above constituents chemically modified with one or more components of the polyhydroxyurethanes and the mixtures of those substances, hereafter named the "Blend of Proteins".

The chemically modified, proteins or peptides with components of the polyhydroxyurethane are obtained following methods described in the literature and the prior art. For example, a modified collagen for the "Blend of Proteins" is obtain by

dissolving collagen in Bis(1,4-aminopropyl) Piperazine, followed by the amidation with a mixture of Carbodiimide (EDC) and N-Hydroxysuccinimide, followed by the precipitation of the modified collagen in TBME (Ter-ButylMethyl Ether), followed by the dissolution and neutralisation of the wet precipitate in a solution of anhydrous Ethanol with 4% of acetic acid, followed by a precipitation of the modified collagen in Ethyl acetate, centrifugation and drying under vacuum. Similarly to the chemical modification of "The GAG Blend" described in the preparation a, the chemically modified protein or peptide can, after its amidation, be further carbamated with one of the cyclic polycarbonate described in the present disclosure in the presence of an acceptable solvent at a temperature between zero and 150° Celsius, preferably between 15 and 80° Celsius. The resulting chemically modified protein can be further carbamated with one of the diamine described in the present disclosure. The successive carbamation of the "Blend of Proteins" can be further proceed to construct a Polyhydroxyurethane side chain of 1 to 25 monomers entities terminated by primary and secondary amine functions on the "Blend of Proteins".

The "Blend of Proteins" is dispersed or dissolved in the continuous media of the preparation b containing an acceptable solvent such as for example, but not limited to water, at a concentration above 1% and optionally monomers or pre-polymers of the polyhydroxyurethane not covalently bound to the "Blend of Proteins", for example a primary or secondary polyamines.

The useful primary or secondary polyamines in the present disclosure are primary or secondary polyamines of ether, ester, ketone, amide, phosphate, sulphate, sulphide, disulfide, alkanes, cycloalkanes, alkenes, cycloalkenes, aryls, substituted or not with hydroxyl functions; or primary or secondary polyamines and primary or secondary polyalkylamines of heterocycles containing one or more oxygen, nitrogen or sulphur atom; or polyetheramines simple or mixed of ethylene glycol and propylene glycol; or a mixture of those compounds.

The pre-polymers of the polyhydroxyurethanes with a Degree of polymerisation from 3 to 200'000, preferably from 3 to 20'000 are obtained following methods described in the literature and the prior art, for example, the method described by Union Carbide Corporation in the patent 3'072'613 dated 8 January 1963. The pre-polymer

of the polyhydroxyurethanes of the preparation b are formulated and prepared by ways ensuring that their terminal endings are primary or secondary amine functions.

Additionally the preparation a can contain Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, the "GAG Blend", chemically modified with pre-

5 polymers of polyhydroxyurethanes or polyhydroxyurethanes, terminated with primary or secondary amine functions.

Additionally the preparation a can contain Glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, the "GAG Blend", chemically modified with monomers or pre-polymers of polyhydroxyurethanes or polyhydroxyurethanes,

10 terminated with primary or secondary amine functions alternatively obtained by amidation. For example, a modified Chondroitin sulphate of the "GAG blend" is obtained by amidation with a diamine or polyamine, such as, but not limited to 1,3-diamino,2-propanol catalysed by a carbodiimide (EDC) and N-Hydroxysuccinimide at room temperature. The degree of amidation is controlled by the catalyst amount. A

15 partially or fully amidated Chondroitin sulphate obtained is purified by successive washing, precipitation and re-suspension in polar and less polar solvents. The partially amidated Chondroitin sulphate can be further amidated with pre-polymers of polyhydroxyurethanes or polyhydroxyurethanes, terminated with primary or secondary amine functions. The reaction is carried at temperature between zero and

20 80° Celsius, preferably, 20 and 60° Celsius with carbodiimide and N-Hydroxysuccinimide catalyst system. The modified glycosaminoglycan is isolated by successive washing, precipitation and re-suspension in polar and less polar solvents.

The preparation b contains the constituents of the disclosure carrying terminal non

25 reacted primary or secondary amine functions from the di and polyamines. The quantity of non reacted primary or secondary amine functions from the di and polyamines from all constituents of the preparation b is sufficient to polymerise or covalently bind to the non reacted cyclic carbonate functions from all constituents of the preparation a to obtain the desired functions from the composition containing

30 polyhydroxyurethanes and glycosaminoglycans, partially hydrolysed Glycosaminoglycans or Glycosamines, chemically modified or not and proteins, partially hydrolysed proteins or peptides, chemically modified or not.

Composition from preparations a and b

The blend of the preparations a and b allows the creation of a homogenous

5 composition containing the constituents of the present disclosure, namely the “GAG blend”, the “Blend of Proteins” in combination with polyhydroxyurethanes or covalently bound with polyhydroxyurethanes.

The useful proportions of the preparations a and b for the blended composition are linked to the desired final application. The molar ratio of cyclic carbonates to
10 amine functions is set between 0,02 and 50. In the example of the substitution or reconstruction of the ECM, those proportions are calculated such as the number of cyclic carbonate functions coming from the preparation a or b are equal or slightly higher than the number of primary and secondary amine functions coming from the preparation b or a.

15 The cyclic carbonate functions from the preparation a or b react with the primary and secondary amine functions from the preparation b or a by polymerisation or covalent bounds to form various Polyhydroxyurethanes. During this mixing of the two preparations, the temperature may increase to temperature between 15 and 150° Celsius depending on the constituents of phase a and b, their concentration and the
20 type of mixing technology chosen from the known technologies. The polymerisation or covalent bounds to form various Polyhydroxyurethanes can additionally be controlled by catalysts such as, but not limited to Zinc, Magnesium, Bismuth, Aluminium salts or covalent compounds or Infra Red photonic sources to control the speed of polymerisation and covalent bounds formation.

25 The obtained composition comprising Polyhydroxyurethanes, Glycosaminoglycans and proteins is for example useful for the preparation of micro-gel or micro-particles to deliver active pharmaceutical substances or to support living cells, or for the treatment of surfaces in contact with living materials, or for the treatment of surfaces
30 to provide them specific affinities to polysaccharides, proteins, partially hydrolysed proteins and peptides , or to chemically modify proteins and partially hydrolysed proteins and peptides to provide them novel physical, biological, pharmacological or

medicinal properties or for the reconstruction and the substitution of extra cellular matrix in vitro or in vivo.

The innovative composition obtained by the mixture of the preparations a and b can be complemented by physiological solution, cells and their growth factors regulators, minerals, particularly phosphate salts and any other component normally present in the ECM environment or for the purpose of the application.

This innovative composition is for example useful for the substitution and the reconstruction of connective, epithelial, nervous, muscular, bones, cartilaginous, or dermal, animal or human extra cellular matrix.

This innovative composition is also useful to coat the surfaces of foreign materials implanted in living organisms for permanent or transitory periods.

In yet another aspect, the present invention provides a composition disclosed herein for coating the surfaces of foreign materials implanted in living organisms for permanent or transitory periods.

In yet another aspect, the present invention provides a composition disclosed herein as carrier, modifier or protector of living cells, biological and pharmacological materials and substances.

Detailed description of the invention

The invention will be better understood in the present chapter, with some examples and the following figures :

Figure 1 shows the viscoelastic rheological properties of a composition according to a first example of the invention.

Figure 2 shows the viscoelastic rheological properties of a composition according to a second example of the invention.

Figure 3 shows the viscoelastic rheological properties of a composition according to a third example of the invention.

Example 1

Preparation a

In a reactor fitted with a mechanical agitator we add 20 parts of Triglycerol (Polyglycerol-3; Solvay), 50 parts of diethyl carbonate and 0,5 part of sodium carbonate finely grounded.

- 5 The blend under agitation is heated over a period of one hour to 90° Celsius, until the mixture appears homogenous. It is then slowly heated to 130° Celsius in one to