

immunogenic, and some of them present antithrombotic effects and interactions with vascular growth factors. Furthermore, due to their plasticity properties, those polysaccharides based hydrogels may be shaped in various forms to allow the design of therapeutic implant or graft biomaterials.

5 For example, Chaouat et al. (Chaouat M, Le Visage C, Autissier A, Chaubet F, Letourneur D. The evaluation of a small-diameter polysaccharide-based arterial graft in rats. *Biomaterials*. 2006 Nov;27(32):5546-53. Epub 2006 Jul 20.) designed a novel polysaccharide based scaffold prepared by using a mixture of pullulan and dextran. Chemical cross-linking of polysaccharides was carried out using the
10 cross-linking agent trisodium trimetaphosphate (STMP). Thereafter, the effectiveness of an arterial material prepared with this scaffold was demonstrated in vivo.

However, despite the advantageous of using polysaccharides for preparing scaffolds as described in Chaouat et al. (2006), the default of porosity of the
15 resulted scaffold remains a drawback to envisage an effective use for therapeutic purposes. Actually, porosity is an essential feature to allow the proliferation, integration and differentiation of the cells inside the scaffold, so that the material can be used as a cell reservoir to reconstruct in vivo the tissue or organ.

Therefore there is still an existing need in the art to develop a method for
20 preparing porous scaffold matrices that can be used for therapeutic purposes.

SUMMARY OF THE INVENTION

Therefore, it is an object of the present ~~invention~~disclosure to provide a method for
25 preparing a porous scaffold which comprises the steps consisting of

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide and one cross-linking agent

b) freezing the aqueous solution of step a)

c) sublimating the frozen solution of step b).

30 characterized in that step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

According to the invention ~~disclosure~~, the term "step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a)" means that the cross-linking of the polysaccharide occurs during the sublimation step (step c)).

It is another object ~~of the present invention~~ to provide a porous scaffold obtainable by the method as above described.

It is still further an object ~~of the present invention~~ to provide the use of porous scaffold of the invention for tissue engineering.

In one aspect, there is provided a method for preparing a porous scaffold which comprises the steps consisting of

- a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide and one cross-linking agent
- b) freezing the aqueous solution of step a)
- c) sublimating the frozen solution of step b)

characterized in that the cross-linking of the polysaccharide occurs during the sublimation step c).

In another aspect, there is provided a porous scaffold obtainable by the method above.

In another aspect, there is provided a vascular substitute made with a scaffold as defined above.

In another aspect, there is provided a cartilage or bone implants made with a scaffold as defined above.

In another aspect, there is provided the use of a scaffold as defined above for the evaluation of the toxicity and/or pharmacology of a product.

In another aspect, there is provided a controlled release system of an active agent made with a scaffold as defined above.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

The term "polysaccharide", as used herein, refers to a molecule comprising two or more monosaccharide units.

The term "alkaline solution", as used herein, refers to a solution having a pH superior to 7.

The term "aqueous solution", as used herein, refers to a solution in which the solvent is water.

The term "cross-linking" refers to the linking of one polymer chain to another one with covalent bonds.

As used herein, a "scaffold" is defined as a semi-solid system comprising a three-dimensional network of one or more species of polysaccharide chains. Depending on the properties of the polysaccharide (or polysaccharides) used, as well as on the nature and density of the network, such structures in equilibrium can contain various amounts of water.

The term "cross-linking agent" includes any agent able to introduce cross-links between the chains of the polysaccharides of the invention.

CLAIMS

1. A method for preparing a porous scaffold which comprises the steps consisting of
 - a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide and one cross-linking agent
 - b) freezing the aqueous solution of step a)
 - c) sublimating the frozen solution of step b).characterized in that step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).
2. The method of claim 1 wherein said polysaccharide is selected from the group consisting of dextran, agar, alginic acid, hyaluronic acid, inulin, pullulan, heparin, chitosan and fucoidan.
3. The method according to claim 1 or 2 wherein said cross-linking agent is selected in the group consisting of trisodium trimetaphosphate (STMP), phosphorus oxychloride (POCl_3), epichlorohydrin, formaldehydes, water soluble carbodiimides, and glutaraldehydes.
4. The method according to claim 3 wherein said cross-linking agent is trisodium trimetaphosphate (STMP)
5. The method according to any one of claims 1 to 4 wherein the aqueous solution of step a) is freeze-dried.
6. The method according to claim 5 wherein the aqueous solution of step a) is freeze-dried under a pressure from 0.1 mBar to 6.5 mBar.
7. The method according to any one of claims 1 to 6 wherein the aqueous solution of step a) is poured in a mould before step b).

8. The method according to any one of claims 1 to 7 wherein said scaffold is shaped.
9. The method according to any one of claims 1 to 8 which comprises a further step consisting of rehydrating the scaffold obtained at step c).
- 5 10. A porous scaffold obtainable by the method according to any one of claims 1 to 9.
11. The porous scaffold of claim 10 wherein the size of the pores is comprised between 1 μm and 500 μm .
- 10 12. The porous scaffold according to claim 10 or 11 wherein the porosity is comprised between 4% and 50%.
13. The porous scaffold according to any one of claims 10 to 12 characterised in that it is loaded with an amount of cells
14. The porous scaffold according to claim 13 wherein the cells are selected in the group consisting of yeast cells, mammalian cells, insect cells, and plant cells.
- 15 15. The porous scaffold according to claim 14 wherein mammalian cells are selected from the group consisting of chondrocytes; fibrochondrocytes; osteocytes; osteoblasts; osteoclasts; muscle cells; synoviocytes; bone marrow cells; mesenchymal cells; epithelial cells, hepatocytes, stromal cells; stem cells; embryonic stem cells; precursor cells derived from adipose tissue; peripheral blood progenitor cells; stem cells isolated from adult tissue; and genetically transformed cells.
- 20 16. The porous scaffold according to any one of claims 10 to 15 for tissue engineering, 3D cell culture or cell delivery for therapeutic use.
- 25 17. A vascular substitute made with a scaffold as defined according to any one of claims 10 to 13.

18. Cartilage or bone implants made with a scaffold as defined according to any one of claims 10 to 13.
19. Use of a scaffold as defined according to any one of claims 10 to 13 for the evaluation of the toxicity and/or pharmacology of a product.
- 5 20. A controlled release system of an active agent made with a scaffold as defined according to any one of claims 10 to 13.

CLAIMS

1. A method for preparing a porous scaffold which comprises the steps consisting of

5 a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide and one cross-linking agent

b) freezing the aqueous solution of step a)

c) sublimating the frozen solution of step b)

characterized in that the cross-linking of the polysaccharide occurs during the sublimation step c).

- 10 2. The method of claim 1 wherein said polysaccharide is selected from the group consisting of dextran, agar, alginic acid, hyaluronic acid, inulin, pullulan, heparin, chitosan and fucoidan.

- 15 3. The method according to claim 1 or 2 wherein said cross-linking agent is selected in the group consisting of trisodium trimetaphosphate (STMP), phosphorus oxychloride (POCl_3), epichlorohydrin, formaldehydes, water soluble carbodiimides, and glutaraldehydes.

4. The method according to claim 3 wherein said cross-linking agent is trisodium trimetaphosphate (STMP).

- 20 5. The method according to any one of claims 1 to 4 wherein the aqueous solution of step a) is freeze-dried.

6. The method according to claim 5 wherein the aqueous solution of step a) is freeze-dried under a pressure from 10 Pa (0.1 mBar) to 650 Pa (6.5 mBar).

7. The method according to any one of claims 1 to 6 which comprises a further step consisting of rehydrating the scaffold obtained at step c).
8. A porous scaffold obtainable by the method according to any one of claims 1 to 7.
- 5 9. The porous scaffold of claim 8 wherein the size of the pores is comprised between 1 μm and 500 μm .
10. The porous scaffold according to claim 8 or 9 wherein the porosity is comprised between 4% and 50%.
- 10 11. The porous scaffold according to any one of claims 8 to 10 characterised in that It is loaded with an amount of cells, preferably selected in the group consisting of yeast cells, mammalian cells, insect cells, and plant cells.
- 15 12. The porous scaffold according to claim 11 wherein the cells are mammalian cells selected from the group consisting of chondrocytes; fibrochondrocytes; osteocytes; osteoblasts; osteoclasts; muscle cells; synoviocytes; bone marrow cells; mesenchymal stem cells; epithelial cells, hepatocytes, stromal cells; stem cells; non human embryonic stem cells; precursor cells derived from adipose tissue; peripheral blood progenitor cells; stem cells isolated from adult tissue; and genetically transformed cells.
- 20 13. The porous scaffold according to any one of claims 8 to 12 for tissue engineering, 3D cell culture or cell delivery for therapeutic use.
14. A vascular substitute made with a scaffold as defined according to any one of claims 8 to 11.
15. Cartilage or bone implants made with a scaffold as defined according to any one of claims 8 to 11.

16. Use of a scaffold as defined according to any one of claims 8 to 11 for the evaluation of the toxicity and/or pharmacology of a product.
17. A controlled release system of an active agent made with a scaffold as defined according to any one of claims 8 to 11.