

BIOLOGICAL MATERIAL SUITABLE FOR THE THERAPY OF  
OSTEOARTHROSIS, LIGAMENT DAMAGE AND FOR THE TREATMENT OF  
JOINT DISORDERS

## 5 FIELD OF THE INVENTION

The present invention regards a biological material  
in viscous liquid form suitable for the therapy of  
osteoarthrosis, tendons, ligaments damage, for the  
10 treatment of joint and connective tissues disorders in  
general, and damaged skin.

### PRIOR ART

The joint cartilage is particularly suitable to  
resist against compression, it neither has blood supply  
15 nor lymphatic drainage and it is entirely free of nerve  
endings. This implies that it is not capable of self-  
regeneration to compensate a surface lesion, unless the  
underlying subchondral layer is not involved.

Thus, if the cartilage lesion is not deep, there will  
20 be no regenerative response. On the contrary, if the  
damage is deep and penetrates into the subchondral bone,  
it triggers a self-reparative process hence the bone  
marrow stem cells start a chondrocyte differentiation  
process which may lead to partial reparation of the  
25 damaged cartilage.

Symptoms, such as pain and swelling of the joint (such as for example the knee), may be the result of a damaged cartilage and the progressive degeneration of the same may develop osteoarthritis.

5        This type of lesion may frequently occur in professional athletes (in that they are more subjected to trauma), or in elderly patients, due to joint trauma and postural defects, or to the normal wear of the cartilage related to the age of the subject.

10        Actually, in the chondral lesions of the knee joint, non-surgical treatments of this type which include physiotherapy and medication, do not allow complete healing of the cartilage defect which instead was attained, with good percentages of success, after the  
15        performance of particularly innovative intra-articular surgical implantation operations.

The main surgical cartilage repair techniques known in the prior art are:

1.        autologous chondrocyte implantation through at  
20        least two different surgical procedures in sequence: the first, less invasive, provides for the collection (by means of arthroscopy) of a normal cartilage tissue of the patient from other non-injured areas of the joint, or from a non-articular cartilage. The collected sample is  
25        thus sent to a laboratory for in vitro cellular

expansion. Once the chondrocytes multiply, there follows the actual surgical operation consisting in grafting, directly in the lesion site, the produced chondrocytes (vehicled in saline solutions) which may be possibly  
5 "fixed in situ" with autologous periosteal tissue. This process is extremely expensive in that it requires 2 surgical operations and the process for in vitro growth of the chondrocytes. Thus, this entails the double admission of the patient in hospital, hence the patient  
10 being subjected twice to anaesthesia and twice to pharmacological treatment.

2. A second simpler method consists in performing perforations on the joint surface to reach the subchondral tissue of the injured area and thus allow the  
15 formation of a mesenchymal blood clot capable of reaching the cartilage defect surface, where the cells may partly differentiate into chondrocytes. However, the newly formed cartilage tissue was mainly fibrous and non-hyaline like the initial cartilage and, thus, it shall  
20 not have the same physical and mechanical characteristics of the native joint.

3. Known for the treatment of osteocartilaginous lesions, is the insertion of surgical implantations or polymer scaffolds containing chondrocytes and/or  
25 mesenchymal stem cells. In this case, the material which

constitutes the scaffold may be semisynthetic polysaccharide such as, for example, a derivative of the hyaluronic acid<sup>(1)</sup>, or consist in collagen matrices. Even this procedure was particularly expensive and required  
5 double admission of patient in hospital and a double operation, as described previously.

Another very common type of lesion and/or inflammation of the connective tissues is the lesion of ligaments or tendons, and in particular that of the  
10 Achilles tendon, especially among professional athletes, generally treated (in its most serious forms) even surgically due to the need of partial or total reconstruction of the injured tissue. Up to date, the surgical techniques used to repair ligaments were based  
15 on the graft of tissues and on synthetic prostheses, which however have a limited efficiency over time. Recent scientific publications have instead proven the capacity of the stem cells to reconstruct the tissue of the damaged tendons and ligaments: mesenchymal cells  
20 collected from the same stem cells introduced into the injured Achilles tendon, were in fact transformed into tenocytes (typical cells of the tendon). The tendon was thus repaired due to an increase of production of collagen which makes the ligaments flexible and  
25 resistant.

Recently, both in maxillofacial and bone surgery<sup>(2)</sup> but also (and above all) in the connective tissues surgery (and particular in the reconstruction/regeneration of tendons/ligaments and skin), the use of platelet-rich hemo-derivatives has become more and more common, in that this type of material is rich in trophic factors such as AGF (Autologous growth factor concentrate), and in particular PDGF-AB and TGF $\beta$  etc <sup>(3), (4)</sup>.

10 These hemo-derivatives are in fact used for stimulating the reparative processes of the damaged skin following to the presence of venous ulcers, mainly in diabetic patients.

The stimulation is normally triggered with different  
15 methodologies, as for example:

- a) mechanical stimulation,
- b) local application of growth factors,
- c) local application of tissue engineering products.

The mechanical stimulation consists in the abrasion  
20 of the bottom and edges of the lesion with a dry sterile gauze or with a bistoury up to bleeding. The local application of growth factor with a platelet concentrate dissolved in plasma allows the release of PDGF (with mitogen and angiogenetic action), TGF-B (for fibroblasts  
25 stimulation) EGF (for epidermal and mesenchymal cells

stimulation) and IGF (as promoter of cellular duplication). The experimentation has demonstrated an increase in tissue vascularization although these hemo-derivatives are difficult to be handled and their  
5 permanence in situ has a very short duration.

The tissue engineering products are more recent and are in the form of heterologous fibroblasts and keratinocytes on a biocompatible support or in the form of autologous fibroblasts on a hyaluronic acid support.

10 This entails the use of surgical practice to take the cells from the patient in order to grow them *in vitro* before loading them on the support.

Considering the previously described state of the art, both regarding the intra-articular or intradermal  
15 administration of the cellular component and the treatment - with platelet products - of the injured regions of tendons/ligaments or skin (both cellular components and platelet rich hemo-derivative being administered by means of special syringes), there arises  
20 the need of providing a "carrier" that is sufficiently fluid but also capable of simultaneously ensuring:

- a good physical/mechanical consistency, to allow the abovementioned administration safeguarding the vitality, the morphology of the cellular membranes and,  
25 simultaneously, the capacity of proliferation and

differentiation of the vehicled cells, but which, in addition,

- allows maintaining the abovementioned cells in the lesion site without requiring further fixing needing  
5 subsequent suturing and medications.

Due to similar reasons, it is necessary to provide a "carrier" capable of safeguarding the integrity of the platelet hemo-derivatives to be used, in order to guarantee all biochemical and enzymatic properties of the  
10 proteins (i.e. the abovementioned trophic factors) contained therein and which, above all, allows maintaining administered active ingredients in the lesion site.

#### SUMMARY OF THE INVENTION.

15 Now, the applicant has discovered that it is possible to overcome the abovementioned drawbacks of the prior art by means of the biological material according to the present invention.

In particular, the biological material according to  
20 the present invention comprises:

a) a liquid carrier comprising a viscous solution containing at least one natural and/or semisynthetic polysaccharide, and having a Dynamic viscosity measured at 20°C and at shear rate of  $D=350 \text{ s}^{-1}$ , comprised between  
25 **100 and 250 cPoise** and/or a Kinematic viscosity comprised

between **99 and 248cSt** (measured at the same conditions);

b) a culture or an extemporaneous preparation of mesenchymal stem cells, and/or

c) a platelet-rich hemo-derivative.

5        This type of material is suitable for the therapy of osteoarthrosis, cartilage damage, tendon damage (in particular the Achilles tendon damage), ligaments and, generally, in the joints and connective tissues disorders in general and damaged skin.

10       In addition, the present invention further relates to pharmaceutical compositions comprising the biological material of the present invention, in particular suitable for intra-articular, intradermal administration, but also for direct application in the lesion site.

15       DETAILED DESCRIPTION OF THE INVENTION.

Relating to the present invention, the term viscous solution is used herein to indicate a homogeneous mixture of two or more components present in which is a solute i.e. the natural and/or semisynthetic polysaccharide  
20       entirely dissolved in a solvent usually water, where the term "water" is used to indicate water for injectable preparations, saline solution, etc.

This type of solution should not be confused with the so-called gel or hydrogel i.e. a semisolid product, whose  
25       components do not dissolve in the solvent, but remain

suspended therein and it is generally made of material which is obtained through the creation of three-dimensional bonds (the so-called crosslinking) of the covalent chemical type, a hydrogen bond or Van der Waals bond between the various components of the gel and/or solvent.

The carrier in liquid form (a) in the biological material according to the present invention is preferably the viscous solution containing the natural and/or semisynthetic polysaccharide.

According to an even more preferred embodiment of the present invention, the carrier (a) in liquid form is the viscous solution essentially consisting of a polysaccharide of natural and/or semisynthetic origin and water.

Regarding the present invention, the expression "essentially consisting of " is used to indicate that a possible third component is present in concentrations comprised between 0.9 and 0.001% on the total weight of the viscous solution.

The polysaccharide of natural origin is preferably selected from hyaluronic acid (HA), cellulose, gellan, chitin, chitosan, pectine or peptic acid, agarose, alginic acid, alginates, starch, polymannans, polyglycans, whose molecular weight as well as the type

of the molecule, is such as to allow/guarantee the formation of a viscous solution (and not a gel) having a Dynamic or Kinematic viscosity comprised in the abovementioned ranges.

5       The polysaccharide of semisynthetic origin is preferably selected from hyaluronic acid derivatives already known to the man skilled in the art such as, for example, the benzyl esters of hyaluronic acid described in EP 216453, its octyl, octadecyl, dodecyl and hexadecyl  
10       amide (EP1095064), and cellulose esters, such as carboxymethylcellulose (CMC) and collagen derivatives. In any case, these natural and semisynthetic polysaccharides must have a molecular weight such that the viscosity thereof is comprised in the abovementioned  
15       ranges, in addition they must exhibit molecular/physical chemical characteristics leading to the formation of a viscous solution and not a gel.

Only this type of viscosity allows obtaining a formulation suitable to be

- 20       • injected intra-articularly or in the lesion sites of tendons and/or ligaments,  
      • intradermally administered in case of cutaneous lesions,

capable both of ensuring the highest vitality of the  
25       cells therein contained, as well as maintaining integer

all the biochemical and enzymatic properties of the trophic factors contained in the platelet-rich hemo-derivatives , possibly present.

Used according to a particularly preferred form of  
5 realization of the invention, are:

- a hyaluronic acid or a pharmaceutically acceptable salt thereof, preferably sodium salt, with average molecular weight comprised between 450 and 730 kDa (medium molecular weight (MW) HA); the concentration  
10 shall be comprised between 5 and 15mg/ml, more preferably 10mg/ml.

- a hyaluronic acid or a pharmaceutically acceptable salt thereof, preferably sodium salt, with average molecular weight comprised between 1000 and 1800 KDa  
15 measured after sterilisation (high molecular weight (MW) HA); the concentration shall be comprised between 2 and 12mg/ml, more preferably between 6 and 8 mg/ml.

- The octylamide of hyaluronic acid, preferably with medium molecular weight(MW), therefore a hyaluronic acid  
20 having an average molecular weight comprised in the aforesaid range between 450 and 730 kDa; the concentration of this amide shall be comprised between 1 and 10mg/ml, more preferably between 2 and 3mg/ml.

- The hexadecylamide of hyaluronic acid, preferably  
25 with medium molecular weight(MW) therefore a hyaluronic

acid having an average molecular weight comprised in the aforesaid range between 450 and 730 kDa; the concentration of this amide shall be comprised between 0,2 and 1,5mg/ml, more preferably between 0,5 and 1mg/ml.

5       • Gellan; the concentration shall be comprised between 2 and 8mg/ml, more preferably 4mg/ml.

      • CMC; the concentration shall be comprised between 15 and 40mg/ml, more preferably 25mg/ml.

Mesenchymal stem cells may be of the autologous and  
10 heterologous type and they are preferably those coming from the bone marrow, peripheral blood, periosteum, umbilical cord or adipose tissue.

For the scope of the present invention, the expression platelet-rich hemo-derivatives is used to  
15 indicate all platelet-rich hemo-derivatives, for example, platelet-rich plasma (PRP), i.e. the supernatant liquid coming from the centrifugation of the venous blood, the platelet concentrate (PC), i.e. the heavier liquid phase coming from the centrifugation of the platelet-rich  
20 plasma, and lastly the platelet gel, i.e. the platelet concentrate which - due to the action of the precipitation agents(such as for example thrombin) - is transformed into gel<sup>(5)</sup>.

Depending on the intended use and the type of lesion,  
25 it is possible to select the type of platelet-rich hemo-

derivative. As a matter of fact, the three products are rich in the abovementioned trophic factors.

The biological material according to the present invention contains components (a) and (b) or (a) and (c),  
5 or the three components (a), (b) and (c).

Furthermore, it is preferable to add the autologous component (c) to the biological material comprising (a) and (b): the abovementioned platelet-rich hemo-derivative comes from the same patient and this product may even be  
10 prepared shortly before the patient is subjected to the abovementioned intra-articular injection or intradermal administration, or before the abovementioned material is applied directly onto the lesion site.

The pharmaceutical compositions according to the  
15 present invention can therefore be used in orthopedy in the (mainly extemporaneous) treatment of cartilage and bone defects (including local use in odontoiatry to favour the plants grip) and can be directly injected in the lesion site of damaged tendons and/or ligaments or  
20 can be used in dermatology both as injectable compositions for an intradermal administration or for topical use for the local treatment of cutaneous ulcers/ lesions difficult to heal/recover.

### **Example 1**

25 Preparation of a viscous solution containing medium

molecular weight HA

1<sup>st</sup> stage: hydration

An amount of the HA sodium salt polysaccharide (500-730KDa MW) equal to 100mg is weighed to prepare a  
5 viscous solution with a final concentration of **10mg/ml**.  
The powders are hydrated with 50% of the required final volume (5ml) using a 0.9% saline solution of Sodium chloride, phosphate buffer or water for injectable preparations, to obtain the desired final concentration.

10 2<sup>nd</sup> stage: solubilisation

The product obtained as described in stage 1, is subjected to magnetic stirring at ambient temperature for at least 1 hr.

The remaining volume (5ml) is subsequently added to  
15 reach the established final concentration, and left under stirring for at least 2 hrs up to complete dissolution of the powders.

The solution thus obtained is subjected to sterilisation by means of an autoclave or UV radiation  
20 and, thus, subjected to the measurement of the Dynamic viscosity by means of a HAAKE RS150 Rheometer, at 20°C, at shear rate of  $D=350 \text{ s}^{-1}$ .

The Dynamic viscosity obtained was of 155cP, thus the product shall have a Kinematic viscosity of 153.6 cSt.

25 **Example 2**

Preparation of a viscous solution containing high molecular weight HA

The process is performed like in example 1 starting from high molecular weight powders of HA to prepare two  
5 viscous solutions with a final concentration of **6mg/ml** and **8 mg/ml** in WFI grade water .

The obtained solutions are sterilised and thus subjected to the measurement of the Dynamic viscosity by means of HAAKE RS150 Rheometer, at 20°C, at shear rate of  
10  $D=350 \text{ s}^{-1}$ .

The Dynamic viscosities obtained were respectively: 158cP and 246cP.

**Example 3**

Preparation of a viscous solution containing gellan

15 The process is performed like in example 1 starting from the powders of gellan to prepare a viscous solution with a final concentration of **4mg/ml** in saline solution.

The solution thus obtained is sterilised and then subjected to the measurement of the Dynamic viscosity by  
20 means of a HAAKE RS150 Rheometer, at 20°C, at shear rate of  $D=350 \text{ s}^{-1}$

The Dynamic viscosity obtained was of 110cP,

**Example 4**

Preparation of a viscous solution containing CMC

25 The process is performed like in example 1 starting

from the powders of CMC to prepare a viscous solution with a final concentration of 25mg/ml in phosphate buffer.

The solution thus obtained is sterilised and then  
5 subjected to the measurement of the Dynamic viscosity by means of a HAAKE RS150 Rheometer, at 20°C, at shear rate of  $D=350 \text{ s}^{-1}$

The Dynamic viscosity obtained was of 220cP,

#### **Example 5**

10 Preparation of a viscous solution containing the octylamide or the hexadecylamide of HA with medium molecular weight (MW)

The process is performed like in example 1 starting from the powders of the octylamide (or from the  
15 hexadecylamide) of medium molecular weight HA, to prepare two viscous solutions with a final concentration of **2mg/ml** and **3mg/ml**, (or of **0,5mg/ml** and **1mg/ml** for the hexadecylamide) in saline solution of sodium chloride 0.9%.

20 The solutions thus obtained (after sterilisation) are subjected to the measurement of Dynamic viscosity by means of a HAAKE RS150 Rheometer, at 20°C, at shear rate of  $D=350 \text{ s}^{-1}$ .

The Dynamic viscosities obtained were respectively:  
25 143cP and 220cP for the octylamide, and 160cP and 230cP

for the hexadecylamide of HA.

Cited bibliography:

<sup>(1)</sup>EP 0863776;

<sup>(2)</sup>"Fattori di crescita autologhi nella chirurgia  
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al, Unità operativa Chirurgia delle Complicanze  
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<sup>(3)</sup>"Different preparation methods to obtain components  
10 as a source of growth factors for local applications" R.  
Zimmermann et al., Transfusion 2001;41:1217-1224.

<sup>(4)</sup>"Platelet-rich plasma preparation using three  
devices: Implications for platelet growth factor release"  
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## CLAIMS

1. A biological material comprising:

- a) a liquid carrier comprising a viscous solution containing at least one natural and/or semisynthetic polysaccharide, and having a Dynamic viscosity measured at 20°C and at shear rate of  $D=350\text{ s}^{-1}$ , comprised between **100 and 250 cPoise** and/or a Kinematic viscosity comprised between **99 and 248cSt** (measured at the same conditions);
- b) a culture or an extemporaneous preparation of mesenchymal stem cells, and/or
- c) a platelet-rich hemo-derivative.

2. The biological material according to claim 1, wherein the carrier in liquid form (a) is a viscous solution comprising the natural and/or semisynthetic polysaccharide.

3. The biological material according to claim 2, wherein said viscous solution consists essentially of the polysaccharide and water.

4. The biological material according to any one of claims 1-3, wherein the polysaccharide of natural origin is selected from the class consisting of: hyaluronic acid, gellan, cellulose, chitin, chitosan, pectine or peptic acid, agarose, alginic acid, alginates, starch, polymannans, and polyglycans.

5. The biological material according to any one of

claims 1-3, wherein the polysaccharide of semisynthetic origin is selected from the class consisting of hyaluronic acid derivatives, collagen derivatives and cellulose derivatives.

5        6. The biological material according to claim 4, wherein the component (a) is hyaluronic acid or a pharmaceutically acceptable salt thereof, with average molecular weight comprised between 450 and 730 kDa.

10       7. The biological material according to claim 5, wherein the component (a) is the hexadecylamide or the octylamide of hyaluronic acid having a medium molecular weight (MW).

15       8. The biological material according to any one of claims 1-7, wherein the mesenchymal stem cells are of the autologous type and they are selected from the class consisting of bone marrow mesenchymal stem cells, peripheral blood mesenchymal stem cells, periosteum mesenchymal stem cells, umbilical cord mesenchymal stem cells or the cells of the adipose tissue.

20       9. The biological material according to any one of claims 1-8, wherein the platelet-rich hemo-derivatives are selected from among platelet-rich plasma, platelet concentrate, and platelet gel.

25       10. The biological material according to any one of claims 1-9, for the therapy of osteoarthrosis, cartilage

damage, tendon damage, ligament damage, in joint disorders, skin lesions or skin ulcers.

11. The biological material according to claim 10, wherein the tendon damage is the Achilles tendon damage.

5 12. The biological material according to any one of claims 1-11, selected from the class consisting of:

biological material containing component (a) and (b),  
biological material containing component (a) and (c),  
biological material containing the three components (a),  
10 (b) and (c).

13. Pharmaceutical compositions comprising the biological material according to any one of claims 1-12, suitable for intra-articular administration, intra-dermal administration, or applied directly in the lesion site.

15 14. Pharmaceutical compositions according to claim 13, wherein the platelet-rich hemo-derivative and/or mesenchymal stem cells are autologous.

15. The pharmaceutical compositions according to anyone of claims 13 and 14, for the treatment of  
20 cartilaginous and bone defects, damaged tendon, damaged ligament or damaged skin.