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(71) Applicant (for all designated States except US): CORPO-
RATION DE L’ECOLE POLYTECHNIQUE DE
MONTREAL [CA/CA]; Campus de l’Universite de
Montreal, 2900 Blvd. Edouard-Montpetit, Ecole Poly-
technique, 2500, Chemin de Polytechnique, Montreal,
Quebec H3T 1J4 (CA).

(72) Inventors; and

(75) Inventors/ Applicants (for US only): OUYANG, Wei
[CA/CA]; 5000 Claramond Apt. 301, Montreal, Quebec
H3X 2S2 (CA).  BUSCHMANN, Michael [CA/CA];
4329 King Edward, Montreal, Quebec H4B 2H4 (CA).
CHEVRIER, Anik [CA/CA]; 24 De Compton Crescent,
Pointe-Claire, Quebec H9R 5V5 (CA).

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(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,
S.R.L.; Suite 2500, 1 place Ville-Marie, Montreal,
Quebec H3B 1R1 (CA).

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MONTREAL [CA/CA]; Campus de l’Universite de
Montreal, 2900 Blvd. Edouard-Montpetit, Ecole Poly-
technique, 2500, Chemin de Polytechnique, Montreal,
Quebec H3T 1J4 (CA).

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(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,
S.R.L.; Suite 2500, 1 place Ville-Marie, Montreal,
Quebec H3B 1R1 (CA).

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Fig. 2

(57) Abstract: The present invention relates to a paste-like polymer composition for use in repairing tissue of a patient which is prepared by combining at least one blood component, a chitosan solution having a chitosan concentration of between about 1.0% w/w to 10.0% w/w and at least one salt, the volume ratio between said blood component and said chitosan solution is between about 4:1 to 1:1.
SPECIFIC BLOOD:CHITOSAN MIXING RATIOS PRODUCING A VISCOUS PASTE-LIKE IMPLANT WITH GOOD HANDLING PROPERTIES FOR TISSUE REPAIR

TECHNICAL FIELD

[0001] The present invention relates to novel viscous paste-like implant with good handling properties for tissue repair by using specific blood/chitosan mixing ratios and physiological chitosan solutions with different concentrations.

BACKGROUND ART

[0002] Chitosan is a linear polysaccharide composed of P-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acylated unit), which primarily results from the alkaline deacetylation of chitin. Chitosan can exist in many structural conformations, depending on a variety of factors that include the degree of hydration, the counterion mixture and the complexity of original chitin mixture. Chitosan and its amino-substituted derivatives are bioerodible, biocompatible and biodegradable cationic polymers that have been advanced for a wide variety of applications, including tissue engineering, drug and gene delivery, pharmaceutical formulation, scaffolds for cell growth and cell encapsulation, wound healing and surface hemostasis.

[0003] A well known property of chitosan is its solubility at acidic pH (<6) and insolubility at neutral pH, making its use in solution with living cells and tissues problematic. Various publications (Chenite, international patent application publication No. WO 99/07416; Chenite et al., 2000, Biomater., 21: 2155-2161; Chenite et al., 2001, Carbohyd. Polym., 46: 39-47) describe that admixing a polyol-phosphate dibasic salt, i.e. glycerol-phosphate (GP), to an aqueous solution of chitosan can increase the pH of the solution while avoiding precipitation of the chitosan. In the presence of these particular salts, chitosan solutions of substantial concentration (0.5-3%) and high molecular weight (> several hundred kDa) remain liquid, at low or room temperature, for a long period of time with physiologically acceptable neutral pH region between 6.8 and 7.2. These chitosan-glycerol phosphate solutions which can gel upon mild heating (for example from 4 to 37°C), while remaining biocompatible,
biodegradable and adhesive to human tissues, and thus provide for new opportunities in the delivery of sensitive therapeutics.

[0004] Many studies previously demonstrated chitosan as being a thrombogenic polymer, e.g. it can accelerate the coagulation of blood. Chitosan-GP solutions were combined with sheep peripheral whole blood to form a thrombogenic mixture that solidified and adhered to a full-thickness cartilage defect in a sheep model. The obtained results showed that solidification of a chitosan-glycerol phosphate/blood implant in microfracture defects improved cartilage repair compared with microfracture alone by increasing the amount of tissue and improving its biochemical composition and cellular organization (Hoemann et al., 2005, J. Bone Joint Surg., 87A: 2671-2686). A bilateral rabbit cartilage repair model was developed to study the effect of chitosan-GP/blood implants on cartilage repair following marrow stimulation. Results showed that chitosan-GP structurally stabilized the blood clots by inhibiting the clot retraction. Treatment of drilled defects with chitosan-GP/blood clots led to the formation of more integrated and hyaline repair tissue above a more porous and vascularised subchondral bone plate compared to drilling alone (Hoemann et al., 2007, Osteoarthritis & Cartilage, 15: 78-89).

[0005] Chitosan-GP/blood implants also increase cell recruitment, transient vascularisation, subchondral remodeling and modulate inflammatory and repair cell phenotype suggesting that these events are in part responsible for increase quantity and quality of repair tissue zone (Chevrier et al., 2007, Osteoarthritis & Cartilage, 15: 316-327; and Hoemann et al., 2010, Am. J. Sports Med., 38, 9: 1845-56). Ultrastructure and compositional detail of chitosan-GP/blood clots, chitosan-GP alone and clots containing whole blood only were investigated by environment scanning electron microscopy (ESEM) in conjunction with energy dispersive X-ray analysis (EDS) (Iliescu et al., 2007, Microsc. Res. Tech., 71: 236-247). It was shown that chitosan formed a network structure in both chitosan-GP gel and chitosan-GP/blood clots. However this structure was altered by aldehyde fixation to produce artifactual aggregates of chitosan microparticles. EDA analysis showed that the majority of glycerol phosphate
can diffuse freely from chitosan-GP gel. Solidification mechanisms of chitosan-
glycerol phosphate/blood implant were investigated as well. Results showed
that chitosan-GP/blood implants solidity through coagulation mechanisms
involving thrombin generation, platelet activation and fibrin polymerization.
Clotting factors can be used to shorten the in situ solidification time of chitosan-
GP/blood implants in microdrilled cartilage defects (Marchand et al., 2009,
Osteoarthritis & Cartilage, 17: 953-960). However, there is still a need in the art
to be provided with more viscous chitosan compositions to facilitate handling
and manipulating.

[0006] There is still a need for an improved blood/chitosan implant. It would
be highly desirable to be provided with blood/chitosan implants that have good
handling properties for tissue repair.

SUMMARY

[0007] In accordance with the present description, there is now provided a
novel viscous paste-like implant with good handling properties for tissue repair.
These viscous paste-like implants are prepared by mixing fresh blood with
physiological chitosan solutions with specific concentrations and at specific mix
ratios.

[0008] According to a first aspect, the present application provides a paste¬
like polymer composition prepared by combining a blood component, a chitosan
solution and at least one salt. In this paste-like composition, the concentration of
chitosan in said chitosan solution is between about 1.0% w/w to 10% w/w; and
the ratio of said blood component to said chitosan solution is between about 4:1
to 1:1 so as to form a paste. In an embodiment, the paste-like polymer further
comprises a mineral acid (including hydrochloric acid, acetic acid, nitric acid,
phosphoric acid, sulfuric acid, boric acid, hydrofluoric acid and/or hydrobromic
acid) or an organic acid. In an embodiment, the at least one salt is an inorganic
salt, such as, for example, sodium salt, chloride salt, potassium salt, calcium
salt, magnesium salt, phosphate salt, sulfate salt and/or carboxylate salt. In yet
a further embodiment, the inorganic salt can be NaCl, KCl, CsCl, CaCl₂, CsF,
KCIO₄, NaNΟ₃ and/or CaSO₄. In another embodiment, the at least one salt is an organic salt, such as, for example, glycerol-phosphate. In a further embodiment, the blood component is whole blood, processed blood, venous blood, arterial blood, blood from bone, blood from bone-marrow, bone marrow, umbilical cord blood and/or placenta blood. In another embodiment, the blood component is human blood. In still another embodiment, the polymer composition has a pH higher than about 6.0 and lower than about 7.8. In another embodiment, the polymer composition has a pH higher than about 6.2 and lower than about 7.8. In still another embodiment, the polymer composition has a pH higher than about 6.2 and lower than about 6.8. In an embodiment, the polymer composition has an osmolality higher than about 200 mOsm/kg and lower than about 600 mOsm/kg. In another embodiment, the osmolality of the polymer composition is higher than about 320 mOsm/kg and lower than about 350 mOsm/kg. In still another embodiment, the osmolality of the polymer composition is higher than about 324 mOsm/kg and lower than about 344 mOsm/kg. In an embodiment, the volume ratio of the blood component-chitosan solution is between 4:1 and 1:1. In another embodiment, the chitosan of the chitosan solution has a degree of deacetylation (DDA) higher than about 20% and lower than about 100%. In still a further embodiment, the DDA is about 81%. In yet another embodiment, the chitosan of the chitosan solution has a number average molecular weight (Mₙ) ranging from about 1 kDa to about 10 MDa, such as, for example, 232 kDa. In still a further embodiment, the concentration of chitosan in said chitosan solution is about 2% w/w.

[0009] According to a second aspect, the present application provides a paste-like polymer composition prepared by combining a blood component, a chitosan solution, a hydrochloric acid solution and a NaCl solution, wherein the ratio of said blood component to said chitosan solution is between about 4:1 to 1:1 so as to form a paste. In an embodiment, the concentration of the chitosan in the chitosan-HCl-NaCl solution is about 2% w/w, the concentration of chitosan-HCl-NaCl is about 50 mM, and/or the concentration of NaCl is about 150 mM. Various embodiments of the blood component, the pH of the paste-like polymer, the osmolality of the paste-like polymer, the volume ratio between the
blood component and the chitosan solution, the degree of deacetylation of the chitosan in the chitosan solution, the molecular weight \( M_n \) of the chitosan in the chitosan solution have been described and do apply herein.

[0010] According to a third aspect, the present application provides a method for repairing a tissue in a subject in need thereof. Broadly, the method comprises the step of introducing into said tissue a paste-like polymer composition as defined herein such that the polymer paste-like composition adheres to the tissue and promotes cell proliferation for repairing the tissue. In an embodiment, the tissue is cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, maxillofacial tissues, temporomandibular tissues, abscesses, resected tumors and/or ulcers.

[0011] According to a fourth aspect, the present application provides the use of a paste-like polymer composition as defined herein for repairing a tissue of a subject, wherein the polymer composition adheres to the tissue and promotes cell proliferation as well as the use of a paste-like polymer composition as defined herein in the manufacture of a medicament for repairing a tissue of a subject, wherein the polymer composition adheres to the tissue and promotes cell proliferation. In an embodiment, the tissue is cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, maxillofacial tissues, temporomandibular tissues, abscesses, resected tumors and/or ulcers.

[0012] According to a fifth aspect, the present application provides a paste-like polymer composition as defined herein for repairing a tissue in a subject, wherein the polymer composition adheres to the tissue and promotes cell proliferation. In an embodiment, the tissue is cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, maxillofacial tissues, temporomandibular tissues, abscesses, resected tumors and/or ulcers.

[0013] According to a sixth aspect, the present application provides a method of preparing a paste-like polymer composition for repairing tissue in a subject. Broadly, the method comprises the steps of dissolving from 1.0% w/w to 10.0% w/w of chitosan in HCl to provide a chitosan-HCl mixture; adding a
NaCl solution to the chitosan-HCl mixture to provide a chitosan-HCl-NaCl mixture; and admixing a blood component to the chitosan-HCl-NaCl, wherein the ratio of said blood component to said chitosan is lower than about 4:1 and higher than about 1:1. In an embodiment, the chitosan is dissolved in HCl by heating at a temperature 60°C. In an embodiment, the concentration of chitosan in the chitosan-HCl-NaCl mixture is about 2% w/w of chitosan, the concentration of hydrochloric acid in the chitosan-HCl-NaCl mixture is about 50 mM, and the mix ratio is about 2:1. Various embodiments of the chitosan molecular weight, the chitosan degree of deacetylation, blood component, the pH of the paste-like polymer composition, the osmolality of the paste-like polymer composition have been described and do apply herein.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0014] Reference will now be made to the accompanying drawings.

[0015] Fig. 1 are photographic representations of the runniness (a means of assessing paste-like handling properties) of rabbit whole blood/chitosan-HCl-NaCl mixtures at different times, wherein the sample numbers from left to right are: 1-1 (1.62% chitosan, mix ratio 3:1); 1-2 (1.62% chitosan, mix ratio 2:1); 2-1 (2.0% chitosan, mix ratio 3:1); 2-2 (2.0% chitosan, mix ratio 2:1); Control (Fresh blood without chitosan); pictures were taken at 30 sec (panel A); 2 min (panel B); 5 min (panel C) and 10 min (panel D).

[0016] Fig. 2 is a histogram of the coagulation time of rabbit whole blood/chitosan-HCl-NaCl mixtures with different chitosan concentrations (1.62% or 2%) at different mix ratios (1:3 or 1:2). Data shown in this figure are averages from n=3 clots.

[0017] Fig. 3 is a histogram showing the mechanical strength of rabbit whole blood/chitosan-HCl-NaCl clots with different chitosan concentrations (1.62% or 2%) at different mix ratios (1:3 or 1:2).

[0018] Fig. 4 are photographic representations of rabbit whole blood/chitosan-HCl-NaCl clots with different chitosan concentrations (1.62% or
2% and mix ratios (1:3 or 1:2) in glass tubes (panels 1, 4, 7, and 10); after removal from the tubes (panels 2, 5, 8 and 11); and after manual crushing for mechanical strength assessment (panels 3, 6, 9 and 12).

[0019] Fig. 5 is a histogram of the coagulation time of human whole blood/chitosan-HCl-NaCl mixtures with different chitosan concentrations (1.62% or 2%) at different mix ratios (1:3 or 1:2) and of fresh blood without chitosan. Data shown in this figure are averages from n=3 clots.

[0020] Fig. 6 is a histogram showing the mechanical strength of human whole blood/chitosan-HCl-NaCl clots with different chitosan concentrations (1.62% or 2%) at different mix ratios (1:3 or 1:2) and of fresh blood without chitosan.

[0021] Fig. 7 are photographic representations of human whole blood/chitosan-HCl-NaCl clots with different chitosan concentrations (1.62% or 2%) and mix ratios (1:3 or 1:2) in glass tubes (panels 1, 4, 7, 10, 13); after removal from the tubes (panels 2, 5, 8, 11 and 14); and after manual crushing for mechanical strength assessment (panels 3, 6, 9, 12 and 15).

DETAILED DESCRIPTION

[0022] It is provided a novel viscous paste-like implant with good handling properties for tissue repair. The novel chitosan preparations comprise a blood component and a salt and are capable of forming a paste.

[0023] These viscous paste-like implants are prepared by mixing fresh blood with physiological chitosan solutions with specific concentrations and at specific mix ratios (e.g. wherein the blood component is present at a volume equal or higher than the chitosan solution, but less than 4 times the volume of the chitosan solution). In a preferred embodiment, a solution containing a specific concentration of chitosan is used to generate the implants (e.g. between 1% to 10% w/w, such as 2% w/w). The mixture described herein has a faster coagulation time, a diminished running distance and possess attractive handling properties, resulting in a more paste-like and viscous mixtures than previous
formulations known in the art. Paste-like mixtures are defined as being thick and viscous.

[0024] It is disclosed herein that a viscous implant can be obtained by mixing blood with chitosan solutions at specific mix ratios (blood component: chitosan solution range from about 4:1 to 1:1). The specific mix ratio are calculated as a volume of a chitosan solution with respect to a volume of a blood component. The concentration of chitosan in the solution, prior to its admixture with the blood component is preferably 2.0 % w/w, but can fluctuate between 1.0% and 10.0% w/w.

[0025] In another embodiment, the chitosan composition is prepared by essentially combining of a blood component, chitosan and at least one salt. The chitosan composition is not prepared with additional components which participates to the formation of the paste or the coagulation of the blood (such as coagulation products for example) but can be prepared with other components such as an acid (to facilitate the dissolution of the chitosan), preservatives, etc.

[0026] The polymer paste-like composition comprises a blood component. Any blood component is contemplated herein, such as whole blood, processed blood, venous blood, arterial blood, blood from bone, blood from bone-marrow, bone marrow, umbilical cord blood or placenta blood. In an embodiment, the blood component is derived from whole blood to be enriched or depleted for a specific blood component. In an embodiment, the volume ratio between the blood component and the chitosan solution is higher than 1:1 but lower than 4:1, such as, for example, 3:1 or 2:1.

[0027] The polymer paste-like composition also comprises chitosan. The chitosan should be able to form a gel and ultimately a thick and viscous paste when combined with blood. Because the chitosan is for use in the treatment of tissue repair, it must also be preferably of physiological grade.
The polymer paste-like composition further comprises at least one salt. Such salt is used for facilitating the dissolution of chitosan in physiological solution. The salt can be an inorganic salt or an organic salt. Inorganic salts include, but are not limited to sodium salt, chloride salt, potassium salt, calcium salt, magnesium salt, phosphate salt, sulfate salt or carboxylate salt, such as, for example, NaCl, KCl, CsCl, CaCl$_2$, CsF, KClO$_4$, NaN0$_3$ or CsSO$_4$. Organic salts include, but are not limited to glycerol-phosphate.

To facilitate the dissolution of chitosan in solution, the paste-like polymer can also include an acid, such as a mineral acid of an organic acid. In a preferred embodiment, HCl is used at a concentration between about 38 to about 50 mM. However other concentrations of HCl can be used.

A preferred embodiment of the current description is described hereinbelow, which is a novel viscous paste-like implant with good handling properties for tissue repair prepared by using specific blood/chitosan mix ratios and physiological chitosan solutions with different concentrations (Table 1). Chitosan (1.62% w/w)-HCl (38 mM)-NaCl (160 mM) solution at pH of 6.56 and osmolality of 344 mOsm/kg, and chitosan (2.0% w/w)-HCl (50 mM)-NaCl (150 mM) solution at pH of 6.49 and osmolality of 324 mOsm/kg were mixed with rabbit whole blood. To prepare the blood/chitosan mixture at a mix ratio of 3:1: a 0.9 ml pipet of fresh blood was added into cryotubes containing 300 μl 1.62% chitosan (or 2.0% chitosan) solution and three 0.39g stainless steel balls, mixed by hand for 10 seconds (the tube being shaken and reversed about 50 times vigorously). 300 μl was transferred into 3 glass tubes at 37°C by using a 1 ml syringe for preparing 3 clots. One clot was used to test coagulation time and fixed immediately after it coagulated; a second clot was used to test coagulation time and fixed at 60 minutes after mixing; the third clot was used to test coagulation time and mechanical strength after 60 minutes. To prepare the blood/chitosan mixture at a mix ratio of 2:1 the procedures described above were repeated by pipetting 0.8 ml of fresh blood into 400 μl chitosan solution. A runniness test to examine handling properties was performed. A total of 5 samples were tested: rabbit whole blood/1.62% chitosan-HCl-NaCl (pH 6.6)
mixture at mix ratios 3:1 and 2:1, rabbit whole blood/2.0% chitosan-HCl-NaCl (pH 6.5) mixture at mix ratios 3:1 and 2:1, and fresh blood without chitosan as a control. A clean plastic board was put on the bench top and tilted at an angle of about 45°. About 0.5 ml of the mixture from each sample was pipetted, then three drops of each sample were carefully placed onto the surface of the board (the distance between each sample being about 2 cm), the runniness of the mixtures was observed and photos were taken at 30 seconds, 2 minutes, 5 minutes and 10 minutes.

[0031] Results from runniness test (Fig. 1) showed that the running distance didn't change significantly from 30 seconds to 10 minutes. The running distance of fresh blood without chitosan was longest among these five samples, indicating quite liquid handling properties. For the same mix ratio, the running distance of the mixtures with higher concentration of chitosan (2.0%) was much shorter than the mixtures with lower concentration of chitosan (1.62%); for the same chitosan concentration, the running distance of mix ratio 3:1 was much longer than mix ratio of 2:1. These results suggest that the use of higher chitosan concentration (2%) and greater ratio of chitosan solution to blood resulted in more attractive handling properties since these mixtures were more paste-like and more viscous. The coagulation time results (Table 2 and Fig. 1) showed that rabbit whole blood/chitosan mixtures at mix ratios of 3:1 and 2:1 can coagulate within 6 minutes. Fresh blood mixed with 1.62% chitosan solution at mix ratio of 3:1 coagulated at 4 minutes; fresh blood mixed with 1.62% chitosan solution at mix ratio of 2:1 coagulated at 3 minutes; fresh blood mixed with 2.0% chitosan solution at mix ratio of 3:1 coagulated at 6 minutes; fresh blood mixed with 2.0% chitosan solution at mix ratio of 2:1 coagulated at 1 minute. This latter composition was particularly useful since its handling properties were much more attractive as it had a more paste-like and more viscous nature that will aid delivery and retention of these implants to challenging sites.

[0032] At the same chitosan concentration, rabbit whole blood mixed with more chitosan solution needed a shorter time to clot (mix ratio of 2:1 < mixing
ratio of 3:1). The mechanical strength test results (Table 3 and Figs. 3 and 4) showed that all four clots were firm and elastic, didn't retract significantly, and there was almost no liquid exuded (scored as +) in 3 of 4 samples. Although the mechanical strength scores were identical for the different chitosan concentrations (1.62% and 2.0%) and different mix ratios (3:1 and 2:1), the mechanical strength of clots prepared with a mix ratio 2:1 were slightly better (1 sample had one hole in the centre of clot and 1 sample broke into 2 connected fragments) than the clots prepared with a mix ratio 3:1 (2 samples broke into 2 fragments).

[0033] Histology results showed that rabbit whole blood/chitosan clots prepared with a mix ratio of 3:1 (both 1.62% and 2% chitosan solution) were homogenous (2 of 2 samples scored +), while the fresh blood/chitosan clots prepared with a mix ratio of 2:1 (both 1.62% and 2% chitosan solution) were not as homogenous (1 sample scored as between ± and - and the other as -) (Table 4).

[0034] In summary, increasing chitosan concentration in chitosan-HCl-NaCl solutions and increasing the ratio of chitosan to blood in the mixture results in significant advantages in terms of a fast coagulation time (as low as 1 minute) and attractive handling properties with a more paste-like and viscous mixtures than previous formulations.

[0035] Similar results were obtained with human whole blood and are shown in Example 2 (see Tables 5-8 and Figs. 5-7).

[0036] The compositions described herein can be used to improve the repair and to regenerate cartilaginous tissues and other tissues including without limitation meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, abscesses, resected tumors, and ulcers.

[0037] There is also contemplated herein the use of the polymer compositions described herein that can be placed or injected into a body site where the mixture aids the repair, regeneration, reconstruction or bulking of
tissues. Repaired tissues include for example without limitation cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, abscesses, resected tumors, and ulcers. The tissue that can be repaired or regenerated is for example without limitation cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissue, abscesses, resected tumors, or ulcers. In some cases, the site of introduction in the body may be surgically prepared to remove abnormal tissues. Such procedure can be done by piercing, abrading or drilling into adjacent tissue regions or vascularized regions to create channels for the polymer composition to migrate into the site requiring repair.

[0038] The present disclosure will be more readily understood by referring to the following examples which are given to illustrate embodiments rather than to limit its scope.

**EXAMPLE 1**

**Formation and preparation of a viscous paste-like homogenous blood/chitosan implant with good handling properties: including runniness test**

1. Preparation of chitosan (1.62% w/w)-HCl (38 mM)-NaCl (160 mM) solution; pH: 6.6; total volume: 10.0 ml

[0039] 0.180 g of chitosan ($M_n$, 232 kDa, 81% DDA) was weighed in a 20 ml beaker, wherein H$_2$O dd was added to the beaker, until the weight of chitosan + H$_2$O = 9.34g. A magnetic stir bar was inserted into the beaker; the solution stirred for about 10 minutes in order to hydrate the chitosan powder as much as possible. 0.38 ml of HCl 1 N (Sigma, Product N° 318949) was added to the solution under moderate stirring. The beaker was covered with parafilm™, and the solution heated to about 60°C for 2 hours, stirred overnight until completely dissolved. 0.32 ml of 5N NaCl (Sigma, Product N° S-9888) solution was added into the beaker and well mixed. The pH of the chitosan solution was physiological at 6.6 and the osmolality was also physiological at 344 mOsm/kg (see Table 1).
2- Preparation of chitosan (2.0% w/w)-HCl (50 mM)-NaCl (150 mM) solution; pH: 6.5; total volume: 10.0 ml

[0040] 0.222 g of chitosan ($M_n$ 232 kDa, 81% DDA) was weighed in a 20 ml beaker and $H_2$O was added to the beaker, until the weight of chitosan + $H_2$O = 9.20g. A magnetic stir bar was inserted into the beaker; the solution stirred for about 10 minutes in order to hydrate the chitosan powder as much as possible. 0.50 ml of HCl 1 N (Sigma, Product N° 318949) was added to the solution under moderate stirring. The beaker was covered with parafilm™, and the solution heated to about 60°C for 2 hours, stirred overnight until completely dissolved. 0.30 ml of 5N NaCl (Sigma, Product N° S-9888) solution was added into the beaker and well mixed. The pH of the chitosan solution was physiological at 6.5 and the osmolality was also physiological at 324 mOsm/kg (Table 1).

| Table 1 |
| Composition and properties of chitosan solutions |
|---|---|---|---|---|---|---|
| Solution No and type. | $C_{\text{chitosan}}$ (%w/w) | $C_{\text{HCl}}$ (mM) | $C_{\text{NaCl}}$ (mM) | $C_{\text{KGP}}$ (%w/w) | Precipitation | pH (Measured) | Osmolality (mOsm/kg) |
| 1- Chitosan-HCl-NaCl pH 6.6 | 1.62 | 38 | 160 | no | no | 6.56 | 344 |
| 2- Chitosan-HCl-NaCl pH 6.5 | 2.0 | 50 | 150 | no | no | 6.49 | 324 |

[0041] Two physiological chitosan-HCl-NaCl solutions containing 1.62% or 2.0% chitosan were successfully prepared: Visually, the solution with the higher chitosan concentration (2.0%) was much more viscous than the solution with the lower chitosan concentration (1.62%).

3- Drawing blood

[0042] Blood was extracted from rabbits using sterile technique. 0.3 cc/kg Hypnorm® was injected IM into rabbits for sedation (for example 0.9 cc for a 3 kg rabbit). First, for each rabbit, ~ 2 ml of blood was collected in a Vacutainer® tube containing EDTA (Fisher, BD, Product N° 02-683-99A) to obtain CBC (complete blood count) and platelet counts. Second, for each rabbit, ~ 5 ml of
blood was collected by using sterile 5cc syringe (Fisher, BD, Product N° 309604) for preparing viscous paste-like blood/chitosan implants.

4- Runniness test of blood/chitosan mixture

To prepare the fresh blood/1.62% chitosan-HCl-NaCl (pH 6.6) mixture (mix ratio: 3:1), 0.9 ml of fresh blood was pipetted into cryotubes containing 300 µl 1.62% chitosan-HCl-NaCl (pH 6.6) solution and three 0.39g stainless steel balls, mixed by hand for 10 seconds (shaken and reversed about 50 times vigorously). To prepare the fresh blood/1.62% chitosan-HCl-NaCl (pH 6.6) mixture (mix ratio: 2:1), 0.8 ml of fresh blood was pipetted into cryotubes containing 400 µl 1.62% chitosan-HCl-NaCl (pH 6.6) solution and three 0.39g stainless steel balls, and mixed by hand for 10 seconds (shaken and reversed about 50 times vigorously). To prepare the fresh blood/2.0% chitosan-HCl-NaCl (pH 6.5) mixture (mix ratio: 3:1 and 2:1), all procedures described hereinabove were repeated by mixing fresh blood with a 2.0% chitosan-HCl-NaCl (pH 6.5) solution.

For runniness tests, a total of 5 samples were tested: samples 1 and 2 were fresh blood/1.62% chitosan-HCl-NaCl (pH 6.6) mixture at mix ratios 3:1 and 2:1; samples 3 and 4 were fresh blood/2.0% chitosan-HCl-NaCl (pH 6.5) mixture at mix ratios 3:1 and 2:1; sample 5 was fresh blood without chitosan as a control. A clean plastic board was tilted on the bench top at an angle of about 45°. About 0.5 ml mixtures from each sample were pipetted, then three drops of each sample was placed carefully onto the surface of the board (the distance between each sample being about 2 cm). The runniness of the mixtures was observed and photos were taken at 30 seconds, 2 minutes, 5 minutes and 10 minutes.

Results from runniness test (see Fig. 1) showed that the running distance of different samples were different, and the running distance didn’t change significantly from 30 seconds to 10 minutes. The running distance of fresh blood without chitosan was longest among these five samples, revealing quite liquid properties. For the same mix ratio, the running distance of the
mixtures with higher concentration of chitosan (2.0%) was much shorter than the mixtures with lower concentration of chitosan (1.62%); for the same chitosan concentration, the running distance of mix ratio 3:1 was much longer than mix ratio 2:1. These results suggest that the use of higher chitosan concentrations (2%) and a greater ratio of chitosan solution to blood (2:1) resulted in more attractive handling properties since these mixtures were more paste-like and more viscous and thus easier to apply in cartilage defects and to other sites that are challenging for delivery.

5- Preparing viscous paste-like blood/chitosan implant (clot), measuring coagulation time and testing mechanical strength of the implant (clot)

[0046] Fresh blood/chitosan-HCl-NaCl mixtures at different chitosan concentrations (1.62% or 2%) and mix ratios (2:1 and 3:1) were prepared as described hereinabove. For each mixture, 300 µl was transferred into 3 glass tubes at 37°C with a 1 ml syringe. Three clots were prepared for each mixture: 1 clot was used to test coagulation time, 1 clot was to test coagulation time and fixed after 60 minutes, 1 clot was used to test coagulation time and mechanical strength after 60 minutes. Coagulation was determined by visualization of the clot at 37°C (all the three glass tubes were used for testing coagulation time). The glass tubes were gently taken from the hot plate vertically every minute, slowly tilted, and the blood mixture was visualized at the bottom of the tube. If the mixture was immobile and formed a clot, it was coagulated; if the mixture was still mobile at the bottom of the tube, it was not yet coagulated. Mechanical strength was tested by putting the clot on the centre of the palm, the clot pressed with a finger until it was crushed, the resistance to compression, liquid expression and crushed appearance observed. The mechanical strength was scored with a 4 "+" system: "+" represents clot was easily broken and crushed appearance was multiple fragments (more than 5 fragments); "++" indicates the clot was relatively firm and the crushed appearance was multiple fragments (3-5 fragments); "+++" represents clot was firm and elastic, crushed appearance was 2-3 fragments; "++++" represents clot was firm and elastic, crushed appearance was 2 fragments (sometimes still connected) or there was just a hole in the center of clot.
The coagulation time results showed that fresh blood/chitosan mixtures at both mix ratios of 3:1 and 2:1 can coagulated within 6 minutes (from 1 minute to 6 minutes). Fresh blood mixed with 1.62% chitosan solution at a mix ratio of 3:1 coagulated at 4 minutes, fresh blood mixed with 1.62% chitosan solution at a mix ratio of 2:1 coagulated at 3 minutes, fresh blood mixed with 2.0% chitosan solution at a mix ratio of 3:1 coagulated at 6 minutes, fresh blood mixed with 2.0% chitosan solution at a mix ratio of 2:1 coagulated at 1 minutes (see Table 2 and Fig. 2). At the same chitosan concentration, fresh blood mixed with more chitosan solution needed less time to clot (mix ratio of 2:1 < mix ratio of 3:1).

Table 2
Coagulation time of blood/Chitosan mixture

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Concentration of chitosan solution and mix ratio</th>
<th>Coagulation time (Min)</th>
<th>Mean value (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blood/chitosan-HCl-NaCl</td>
<td>1.62% and 3:1 (clot 1-1)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.62% and 2:1 (clot 1-2)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0% and 3:1 (clot 2-1)</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0% and 2:1 (clot 2-2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

The mechanical strength test results showed that all four clots were firm and elastic. For clot 1-1 (1.62%, 3:1), almost no liquid exuded (scored as +), the clot didn't retract, the mechanical strength was "++++", after crushing, the clot broke in two fragments. For clot 1-2 (1.62%, 2:1), some liquid exuded (scored as ++), the clot didn't retract significantly, the mechanical strength was "++++", and after crushing, the clot had just a hole in the centre. For clot 2-1 (2.0%, 3:1), almost no liquid exuded (scored as +), the clot didn't retract, the mechanical strength was "++++", after crushing, the clot broke in two fragments. For clot 2-2 (2.0%, 2:1), almost no liquid exuded (scored as +), the clot didn't retract, the mechanical strength was "++++", after crushing, the clot broke into
two connected fragments (see Table 3, Fig. 3 and Fig. 4). Although the mechanical strength scores were identical for the different chitosan concentrations (1.62% and 2.0%) and different mix ratios (3:1 and 2:1), the mechanical strength of clots prepared with a mix ratio of 2:1 was slightly better (1 sample had just a hole in the centre of clot and 1 sample broke into 2 connected fragments) than the clots prepared with a mix ratio of 3:1 (2 samples broke into 2 fragments).

Table 3
Mechanical test of blood clots

<table>
<thead>
<tr>
<th>Sample</th>
<th>Resistance to compression</th>
<th>Liquid expressed</th>
<th>Crushed appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blood/chitosan-HCl-NaCl clot 1-1 (1.62%, 3:1)</td>
<td>Firm and elastic ++++</td>
<td>Almost no liquid expressed +</td>
<td>2 fragments</td>
</tr>
<tr>
<td>Fresh blood/chitosan-HCl-NaCl clot 1-2 (1.62%, 2:1)</td>
<td>Firm and elastic ++++</td>
<td>++</td>
<td>Hole in center</td>
</tr>
<tr>
<td>Fresh blood/chitosan-HCl-NaCl clot 1-1 (2.0%, 3:1)</td>
<td>Firm and elastic ++++</td>
<td>Almost no liquid expressed +</td>
<td>2 fragments</td>
</tr>
<tr>
<td>Fresh blood/chitosan-HCl-NaCl clot 1-1 (2.0%, 2:1)</td>
<td>Firm and elastic ++++</td>
<td>Almost no liquid expressed +</td>
<td>2 fragments but still connected</td>
</tr>
</tbody>
</table>

6- Histological treatment and homogeneity evaluation of blood/chitosan clots

[0049] Fixed clots were sectioned in 2 parts. One part was embedded in paraffin, stained with Safranin O/Fast Green and observed by optical microscopy. One part was stored at 4°C until further use. All the photos were taken from the blood/chitosan clot samples fixed 60 minutes after the clots were prepared; two photos taken with 5x and 40x objectives from different regions of each sample were used for homogeneity evaluation. Each specimen was observed under microscopy with special emphasis on: presence of bubbles or cracks; presence and distribution of precipitates of chitosan described as large aggregates or small aggregates; chitosan distribution and whether or not they are homogeneously dispersed across the section; erythrocyte morphology in term of discoid, shrunken, swollen or chaining.
The histology results showed that the fresh blood/chitosan clots prepared with a mix ratio of 3:1 (both 1.62% and 2% chitosan solution) were homogenous (both scored as "+"). The fresh blood/chitosan clots prepared with a mix ratio of 2:1 (both 1.62% and 2% chitosan solution) were not as homogenous (one scored as "between ± and -" and the other as "). (see Fig. 4 and Table 4).

Table 4
Comparison of homogeneity for blood/chitosan clots with different chitosan and mixing ratio

<table>
<thead>
<tr>
<th>Clot-1 samples (1.62% chitosan solution)</th>
<th>Homogeneity of clot-1</th>
<th>Clot-2 samples (2.0% chitosan solution)</th>
<th>Homogeneity of clot-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood 1-1-60 (3:1)</td>
<td>+</td>
<td>PRP 2-1-60 (3:1)</td>
<td>+</td>
</tr>
<tr>
<td>blood 1-2-60 (2:1)</td>
<td>- (between ± and -)</td>
<td>PRP 2-2-60 (2:1)</td>
<td>-</td>
</tr>
</tbody>
</table>

EXAMPLE 2
Formulation and characterization of fresh human blood/chitosan clots by using chitosan solutions with different concentration at different mix ratios

1- Preparation of chitosan (1.62% w/w)-HCl (38 mM)-NaCl (160 mM) solution; pH: 6.6; total volume: 10.0 ml

The chitosan-HCl-NaCl solution ($M_n$ 232 kDa, 81% DDA) was prepared as described hereinabove. The pH of the chitosan solution was physiological at 6.6 and the osmolality was also physiological at 317 mOsm/kg (Table 5).

2- Preparation of chitosan (2.0% w/w)-HCl (50 mM)-NaCl (150 mM) solution; pH: 6.5; total volume: 10.0 ml

The chitosan-HCl-NaCl solution ($M_n$ 232 kDa, 81% DDA) was prepared as described hereinabove. The pH of the chitosan solution was physiological at 6.5 and the osmolality was also physiological at 351 mOsm/kg (Table 5).
Table 5
Composition and properties of chitosan solutions

<table>
<thead>
<tr>
<th>Solution No and type.</th>
<th>(C_{\text{chitosan}}) (%w/w)</th>
<th>(C_{\text{HCl}}) (mM)</th>
<th>(C_{\text{NaCl}}) (mM)</th>
<th>(C_{\text{BGF}}) (%w/w)</th>
<th>Precipitation</th>
<th>(\text{pH}) (Measured)</th>
<th>Osomolality (mOsm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chitosan-HCl-NaCl pH6.6</td>
<td>1.62</td>
<td>38</td>
<td>160</td>
<td>no</td>
<td>no</td>
<td>6.56</td>
<td>317</td>
</tr>
<tr>
<td>2. Chitosan-HCl-NaCl pH6.5</td>
<td>2.0</td>
<td>50</td>
<td>150</td>
<td>no</td>
<td>no</td>
<td>6.49</td>
<td>351</td>
</tr>
</tbody>
</table>

[0053] Two physiological chitosan-HCl-NaCl solutions containing 1.62% or 2.0% chitosan were successfully prepared. Visually, the solution with the higher chitosan concentration (2.0%) was much more viscous than the solution with the lower chitosan concentration (1.62%).

3- Drawing blood

[0054] About 12 cc whole blood was extracted from a donor with informed consent using a blood-taking IRB protocol. First, ~ 2 ml of blood was collected in a Vacutainer™ tube containing EDTA to obtain CBC (complete blood count) and platelet count. Second, ~ 5ml of blood was collected with a sterile 5cc syringe (Fisher, BD, Product N° 309604) for preparing fresh blood/chitosan clots.

4- Formulation and characterization of fresh blood/chitosan clots by using chitosan-NaCl solutions with different concentration at different mixing ratio

[0055] Fresh human whole blood/chitosan-HCl-NaCl mixtures at different chitosan concentrations (1.62% or 2%) and mix ratios (2:1 and 3:1) were prepared as described hereinabove.

[0056] Coagulation of the clots was determined by visualization of the clot at 37°C as described previously. Mechanical strength was tested as described hereinabove.

[0057] The coagulation time results showed that human whole blood/chitosan mixtures at both mix ratios of 3:1 and 2:1 coagulated within 10 minutes, faster than fresh blood without chitosan. Fresh blood mixed with 1.62%
chitosan solution at a mix ratio of 3:1 coagulated at 9.3 minutes, fresh blood mixed with 1.62% chitosan solution at a mix ratio of 2:1 coagulated at 8.7 minutes, fresh blood mixed with 2.0% chitosan solution at a mix ratio of 3:1 coagulated at 6.3 minutes, fresh blood mixed with 2.0% chitosan solution at a mix ratio of 2:1 coagulated at 5 minutes. Fresh blood without chitosan (control sample) coagulated at 10.3 minutes (Table 6 and Fig. 5).

[0058] At the same chitosan concentration, human whole blood mixed with more chitosan solution needed less time to clot (mix ratio of 2:1 < mix ratio of 3:1). At the same mix ratio, human whole blood mixed with high concentration chitosan solution needed less time to clot (2.0% < 1.62%).

Table 6
Coagulation time of human whole blood/chitosan mixtures

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Concentration of chitosan solution and mix ratio</th>
<th>Coagulation time (Min)</th>
<th>Mean value (Min)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blood/Chitosan-HCl-NaCl</td>
<td>1.62% and 3:1 (clot 2-1-1)</td>
<td>1 10</td>
<td>9.3</td>
<td>All the mixture samples coagulated within 10 minutes (from 5 minutes to 10 minutes); for using chitosan with same concentration, fresh blood mixed with more chitosan solution need shorter time to form clot (mixing ratio of 2:1 &lt; mixing ratio of 3:1); for using chitosan with same mixing ratio, fresh blood mixed with high concentration chitosan solution need shorter time to form clot (2.0% &lt; 1.62%).</td>
</tr>
<tr>
<td></td>
<td>1.62% and 2:1 (clot 2-1-2)</td>
<td>2 9</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0% and 3:1 (clot 2-2-1)</td>
<td>1 6</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0% and 2:1 (clot 2-2-2)</td>
<td>1 5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Without chitosan (clot-C)</td>
<td>1 11</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[0059] The mechanical strength test results showed that all the clots were firm and elastic. For clot 2-1-1 (1.62%, 3:1), almost no liquid exuded (scored as +), the clot didn’t retract, the mechanical strength was "++++", after crushing, the clot had just a hole in the centre. For clot 2-1-2 (1.62%, 2:1), almost no liquid exuded (scored as +), the clot didn’t retract, the mechanical strength was "++++", after crushing, the clot broke into two fragments. For clot 2-2-1 (2.0%, 3:1), almost no liquid exuded (scored as +), the clot didn’t retract, the
mechanical strength was "++++", after crushing, the clot broke in two connected fragments. For clot 2-2-2 (2.0%, 2:1), some liquid exuded (scored as ++), the clot didn't retract significantly, the mechanical strength was "++++", after crushing, the clot broke in two connected fragments. For clot-C (blood without chitosan), some liquid exuded (scored as ++), the clot retracted to about 75% of initial size, the mechanical strength was "++++", after crushing, the clot broke in two connected fragments (Table 7, Figs. 6 and 7).

Table 7
Mechanical strength test of human whole blood/chitosan clots

<table>
<thead>
<tr>
<th>Sample</th>
<th>Resistance to compression</th>
<th>Liquid expressed</th>
<th>Crushed appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blood/Chitosan-HCl-NaCl clot 2-1-1</td>
<td>Firm and elastic ++</td>
<td>Almost no liquid expressed +</td>
<td>Hole in center</td>
</tr>
<tr>
<td>(1.62%, 3:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh blood/Chitosan-HCl-NaCl clot 2-1-2</td>
<td>Firm and elastic ++</td>
<td>Almost no liquid expressed +</td>
<td>2 fragments</td>
</tr>
<tr>
<td>(1.62%, 2:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh blood/Chitosan-HCl-NaCl clot 2-2-1</td>
<td>Firm and elastic ++</td>
<td>Almost no liquid expressed +</td>
<td>2 fragments but still connected</td>
</tr>
<tr>
<td>(2.0%, 3:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh blood/Chitosan-HCl-NaCl clot 2-2-2</td>
<td>Firm and elastic ++</td>
<td>++</td>
<td>2 fragments but still connected</td>
</tr>
<tr>
<td>(2.0%, 2:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh blood without chitosan clot-C</td>
<td>Firm and elastic ++</td>
<td>++</td>
<td>2 fragments but still connected</td>
</tr>
</tbody>
</table>

5- Histological treatment and homogeneity evaluation of PRP/chitosan clots

[0060] Histological treatment and homogeneity evaluation was performed as described previously.

[0061] The histology results showed that the human whole blood/chitosan clots prepared with a chitosan concentration of 1.62% (both mix ratios 2:1 and 3:1) were homogenous (both scored as "+"). The fresh blood/chitosan clots prepared with a chitosan concentration of 2% (both mix ratios 2:1 and 3:1) were not as homogenous (both scored as "-") (Table 8).
Table 8

Homogeneity of blood/chitosan clots.

<table>
<thead>
<tr>
<th>Clot-1 samples (1.62% chitosan solution)</th>
<th>Homogeneity of clot-1</th>
<th>Clot-2 samples (2.0% chitosan solution)</th>
<th>Homogeneity of clot-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood 2-1-1 (3:1)</td>
<td>+</td>
<td>blood 2-2-1 (3:1)</td>
<td>-</td>
</tr>
<tr>
<td>blood 2-1-2 (2:1)</td>
<td>+</td>
<td>blood 2-2-2 (2:1)</td>
<td>-</td>
</tr>
<tr>
<td>blood clot-C (without chitosan)</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[0062] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.
WHAT IS CLAIMED IS:

1- A paste-like polymer composition prepared by combining a blood component, a chitosan solution and at least one salt, wherein:

- the concentration of chitosan in said chitosan solution is between about 1.0% w/w to 10% w/w; and
- the ratio of said blood component to said chitosan solution is between about 4:1 to 1:1 so as to form a paste.

2- The paste-like polymer composition of 1, further comprising a mineral acid or an organic acid.

3- The paste-like polymer composition of claim 1 or 2, wherein the at least one salt is an inorganic salt.

4- The paste-like polymer composition of claim 3, wherein the inorganic salt is sodium salt, chloride salt, potassium salt, calcium salt, magnesium salt, phosphate salt, sulfate salt or carboxylate salt.

5- The paste-like polymer composition of claim 3, wherein the inorganic salt is NaCl, KCl, CsCl, CaCl₂, CsF, KClO₄, NaN₃ or CaSO₄.

6- The paste-like polymer composition claim 1 or 2, wherein the at least one salt is an organic salt.

7- The paste-like polymer composition of claim 6, wherein the organic salt is glycerol-phosphate.

8- The paste-like polymer composition of any one of claims 1-7, wherein the blood component is whole blood, processed blood, venous blood, arterial
blood, blood from bone, blood from bone-marrow, bone marrow, umbilical cord blood or placenta blood.

9- The paste-like polymer composition of any one of claims 1-8, wherein the blood component is human blood.

10- The paste-like polymer composition of any one of claims 1-9, wherein the polymer composition has a pH between 6.0 and 7.8.

11- The paste-like polymer composition of claim 10, wherein the polymer composition has a pH between 6.4 and 7.8.

12- The paste-like polymer composition of claim 10, wherein the polymer composition has a pH between 6.4 and 6.6.

13- The paste-like polymer composition of any one of claims 1-12, wherein the polymer composition has an osmolality between 200 mOsm/kg and 600 mOsm/kg.

14- The paste-like polymer composition of claim 13, wherein the osmolality is between 320 mOsm/kg and 350 mOsm/kg.

15- The paste-like polymer composition of claim 13, wherein the osmolality is between 324 mOsm/kg and 344 mOsm/kg.

16- The paste-like polymer composition of any one of claim 1-15, wherein the mineral acid is hydrochloric acid, acetic acid, nitric acid, phosphoric acid, sulfuric acid, boric acid, hydrofluoric acid or hydrobromic acid.

17- The paste-like polymer composition of any one of claims 1-16, wherein the ratio is higher than or equal to about 3:1.

18- The paste-like polymer composition of any one of claims 1-16, wherein the ratio is higher than or equal to 2:1.
19- The paste-like polymer composition of any one of claims 1-18, wherein the chitosan of said chitosan solution has a degree of deacetylation (DDA) between 20% to 100%.

20- The paste-like polymer composition of claim 19, wherein the DDA is 81%.

21- The paste-like polymer composition of any one of claims 1-20, wherein the chitosan of the chitosan solution has a number average molecular weight (M_n) ranging from 1 kDa to 10 MDa.

22- The paste-like polymer composition of claim 21, wherein the M_n is 232 kDa.

23- The paste-like polymer composition of any one of claims 1-22, wherein the concentration of chitosan in said chitosan solution is about 2% w/w.

24- A paste-like polymer composition prepared by combining a blood component, a chitosan solution, hydrochloric acid solution and a NaCl solution, wherein the ratio of said blood component to said chitosan solution is between about 4:1 to 1:1 so as to form a paste.

25- The paste-like polymer composition of claim 24, wherein the concentration of chitosan in the chitosan-HCl-NaCl solution is about 2% w/w.

26- The paste-like polymer composition of claim 24 or 25, wherein the concentration of hydrochloric acid in said chitosan-HCl-NaCl solution is about 50 mM.

27- The paste-like polymer composition of any one of claims 24-26, wherein the concentration of NaCl in said chitosan-HCl-NaCl solution is about 150 mM.
28- The polymer composition of any one of claims 24-27, wherein the blood component is whole blood, processed blood, venous blood, arterial blood, blood from bone, blood from bone-marrow, bone marrow, umbilical cord blood or placenta blood.

29- The paste-like polymer composition of any one of claims 24-28, wherein the blood component is human blood.

30- The paste-like polymer composition of any one of claims 24-29, wherein the polymer composition has a pH between 6.0 and 7.8.

31- The paste-like polymer composition of claim 30, wherein the polymer composition has a pH between 6.2 and 7.8.

32- The paste-like polymer composition of claim 30, wherein the polymer composition has a pH between 6.2 and 6.8.

33- The paste-like polymer composition of any one of claims 24-32, wherein the polymer composition has an osmolality between 200 mOsm/kg and 600 mOsm/kg.

34- The paste-like polymer composition of claim 33, wherein the osmolality is between 320 mOsm/kg and 350 mOsm/kg.

35- The paste-like polymer composition of claim 33, wherein the osmolality is between 324 mOsm/kg and 344 mOsm/kg.

36- The paste-like polymer composition of any one of claims 24-35, wherein the ratio is about 3:1.

37- The paste-like polymer composition of any one of claims 24-35, wherein the ration is about 2:1.
The paste-like polymer composition of any one of claims 24-37, wherein the chitosan of said chitosan solution has a degree of deacetylation (DDA) between 20% to 100%.

The paste-like polymer composition of claim 38, wherein the DDA is 81%.

The paste-like polymer composition of any one of claims 24-39, wherein the chitosan of said chitosan solution has a number average molecular weight ($M_n$) ranging from 1 kDa to 10 MDa.

The paste-like polymer composition of claim 40, wherein the $M_n$ is 232 kDa.

A method for repairing a tissue in a subject in need thereof, said method comprising the step of introducing into said tissue a paste-like polymer composition as defined in any one of claims 1-41 such that the polymer paste-like composition adheres to the tissue and promotes cell proliferation for repairing the tissue.

The method of claim 42, wherein said tissue is selected from the group consisting of cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, maxillofacial tissues, temporomandibular tissues, abscesses, resected tumors and ulcers.

Use of a paste-like polymer composition as defined in any one of claims 1-41 for repairing a tissue of a subject, wherein the polymer composition adheres to the tissue and promotes cell proliferation.

Use of a paste-like polymer composition as defined in any one of claims 1-41 in the manufacture of a medicament for repairing a tissue of a subject, wherein the polymer composition adheres to the tissue and promotes cell proliferation.
46- The use of claim 44 or 45, wherein said tissue is selected from the group consisting of cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, maxillofacial tissues, temporomandibular tissues, abscesses, resected tumors and ulcers.

47- A paste-like polymer composition as defined in any one of claims 1-41 for repairing a tissue in a subject, wherein the polymer composition adheres to the tissue and promotes cell proliferation.

48- The polymer composition of claim 47, wherein said tissue is selected from the group consisting of cartilage, meniscus, ligament, tendon, bone, skin, cornea, periodontal tissues, maxillofacial tissues, temporomandibular tissues, abscesses, resected tumors and ulcers.

49- A method of preparing a paste-like polymer composition for repairing tissue in a subject, said method comprising the steps of:

   a) dissolving from 1.0% w/w to 10.0% w/w of chitosan in HCl to provide a chitosan-HCl mixture;

   b) adding a NaCl solution to the chitosan-HCl mixture to provide a chitosan-HCl-NaCl mixture; and

   c) admixing a blood component to the chitosan-HCl-NaCl, wherein the ratio of said blood component to said chitosan is between about 4:1 to about 1:1 so as to form the paste-like polymer composition.

50- The method of claim 49, wherein the chitosan is dissolved in HCl by heating at a temperature 60°C.

51- The method of claim 49 or 50, wherein the concentration of chitosan in the chitosan-HCl-NaCl mixture is about 2% w/w of chitosan, the
concentration of hydrochloric acid in the chitosan-HCl-NaCl mixture is about 50 mM, and the ratio is about 2:1.

52- The method of any one of claims 49-51, wherein the chitosan of the chitosan:HCl mixture has a degree of deacetylation (DDA) of between about 20% to about 100%.

53- The method of any one of claims 49-52, wherein the chitosan of the chitosan:HCl mixture has a number average molecular weight ($M_n$) between about 1 kDa to about 10 MDa.

54- The method of any one of claims 49-52, wherein the blood component is whole blood, processed blood, venous blood, arterial blood, blood from bone, blood from bone-marrow, bone marrow, umbilical cord blood or placenta blood.

55- The method of any one of claims 49-54, wherein the blood component is human blood.

56- The method of any one of claims 49-55, wherein the polymer composition has a pH between 6.0 and 7.8.

57- The method of claim 56, wherein the polymer composition has a pH between 6.2 and 7.8.

58- The method of claim 56, wherein the polymer composition has a pH between 6.2 and 6.8.

59- The method of any one of claims 49-58, wherein the polymer composition has an osmolality between 200 mOsm/kg and 600 mOsm/kg.
60- The method of claim 59, wherein the osmolality is between 320 mOsm/kg and 350 mOsm/kg.

61- The method of claim 59, wherein the osmolality is between 324 mOsm/kg and 344 mOsm/kg.

62- The method of claim 52, wherein the DDA is 81%.

63- The method of claim 53, wherein the $M_n$ is 232 kDa.
Coagulation time of blood/chitosan clots

Fig. 2
Mechanical strength of blood/chitosan clots

Fig. 3
Fig. 5
Fig. 6
Fig. 7
INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA20 10/00 1843

A. CLASSIFICATION OF SUBJECT MATTER
IPC: C08L 5/08 (2006.01) , A61K 35/14 (2006.01) , C08K 11/00 (2006.01) , C08K 3/00 (2006.01) , C08K 5/21 (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: C08L 5/08 (2006.01) , A61K 35/14 (2006.01) , C08K 11/00 (2006.01) , C08K 3/00 (2006.01) , C08K 5/21 (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Intellect

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
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[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex.

** Special categories of cited documents:
“X” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
“Y” document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
“Z” document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Date of the actual completion of the international search 31 January 2011 (31-01-2011)
Date of mailing of the international search report 11 February 2011 (11-02-2011)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer
Rebecca Gardner (819) 956-4 117
### Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claim Nos.: 42 and 43
   because they relate to subject matter not required to be searched by this Authority, namely:
   
   Claims 42 and 43 are directed to a method for treatment of the human or animal body by surgery or therapy, are not required to be searched nor is a written opinion required by this Authority. Regardless, this Authority has established a written opinion based on the alleged effect or purpose/use of the product defined in claims 42 and 43.

2. [X] Claim Nos.: 1-63 (in part)
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
   
   The inclusion of the term "blood component" and "salt" in the claims causes ambiguity as it fails to clearly and explicitly define the scope of the claims. The above terms attempt to encompass the specific example(s) as well as including those not contemplated by the applicant. The present description only enables the use of whole blood in combination with chitosan-HCl-NaCl.

3. [ ] Claim Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.
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