Abstract:
The present invention relates to a new platelet-rich plasma preparation, a method of preparation thereof, a use thereof, a device for the preparation thereof and preparations containing such a platelet-rich plasma preparation. Specifically, the invention provides platelet-rich plasma preparations for use in tissue regeneration and bone regeneration.
PROCESS AND DEVICE FOR THE PREPARATION OF PLATELET RICH PLASMA FOR EXTEMPORANEOUS USE AND COMBINATION THEREOF WITH SKIN AND BONE CELLS

Field of the invention

The present invention is related to the field of tissue regeneration and especially skin and bone regeneration. It concerns more particularly a new platelet-rich plasma preparation as a biological scaffold, a method of preparation thereof, a use thereof, a device for the preparation thereof and preparations containing such a platelet-rich plasma preparation for extemporaneous use.

Background of the invention

The importance of biological autologous materials in the healing process has been well documented. Most importantly, two biological autologous materials have been shown to be directly implicated in the formation of the structure of blood clots, which provide a haemostatic barrier whose role is to ensure hemostasis and seal the wound:

(1) fibrin, which derives from the separation of plasma fibrinogen into two strands through the action of thrombin, and (2) the activated membranes of platelets.

The wound healing process is generally presented as the succession of a coagulation phase, an inflammatory process and a regeneration process.

The coagulation phase (blood clotting or clot formation) is a complex process whereby a damaged blood vessel wall is covered by a fibrin clot to stop hemorrhage and the repair of the damaged vessel is initiated by the release in large quantities of cytokines and growth factors from platelet alpha granules. The formation of blood clots (formed in physiological conditions by fibrin, platelets and red blood cells, among other blood components) is a natural phenomenon that results from tissue trauma and its role in the wound healing process, as well as in the union of bone fractures, is well-known.

The inflammation process, which follows the formation of a blood clot, is stimulated by numerous vasoactive mediators and chemotactic factors (specific signals in the form of proteins) released by white blood cells and platelets. These signals attract macrophages that "clean" the site from bacteria and foreign particles as well as red blood cells before the migration of new cells.
The tissue regeneration phase involves the chemoattraction and the mitosis of the undifferentiated cells in the scaffold (or growth matrix) formed by the blood clot. The new cells which multiply under the stimulation of platelet growth factors will replace damaged or destroyed cells ingested by macrophages.

Growth factors and numerous plasma proteins, also called signaling molecules, which promote cell migration and division within blood clots, play a crucial role in the wound healing process.

Theoretically, it is possible to amplify the effects of these first phases in the wound-healing cascade by discarding the red blood cells and increasing the concentration of growth factors.

Blood clotting amplification can be defined as the formation of an "enriched clot (EC)". ECs are obtained through the use of platelet concentrates and have been described in *Platelets and Megacaryocytes 2004, vol 1 & 2, as "Structure and signals", Ed. Gibbins and Mahaut-Smith, Humana Press, New Jersey.*

Platelet-rich plasma (PRP), can be defined as an autologous concentrate of platelets in a small volume of plasma; it has been developed as an autologous biomaterial and has proven to be useful in the healing and regeneration of tissues *(Marx et al, 2004, *J. Oral Maxillofac. Surg.,* 62, 489-496)*. PRP not only consists in a platelet concentrate but also contains growth factors (such as platelet-derived growth factor: PDGF, vascular endothelial growth factor: VEGF, transforming growth factor: TGF and epidermal growth factor: EGF) that are actively secreted by platelets and are known to have a fundamental role in wound healing initiation process.

For example, PDGF is known to initiate connective tissue healing, including bone regeneration and repair. PDGF also increases mitogenesis (healing cells), angiogenesis (endothelial mitosis into functioning capillaries) and macrophage activation. VEGF released by the leukocytes is also known to have potent angiogenic, mitogenic and vascular permeability-enhancing activities on endothelial cells. TGF-b promotes cell mitosis and differentiation for connective tissue and bone, acts on mesenchymal stem cells, preosteoblasts and fibroblasts and inhibits osteoclast formation. EGF is known to induce epithelial development and promote angiogenesis.
Platelet concentrates are generally used in dental implantology and bone surgery, notably in the USA. Various techniques of preparation of PRP by centrifugation processes have been developed. However, due to the sensitivity of the platelet cells and the variability of the efficiency of the methods of separation of the platelets from the red blood cells, a great variability exist among the methods used for the preparation of platelet concentrates (Marx et al., 2004, above; Roukis et al., Adv. Ther., 2006, 23(2):218-37).

It has been recently demonstrated that the positive effects of platelet-rich plasma on bone regeneration spans a limited range of platelet concentration and revealed that an inhibitory effect occurs in the presence of more than $10^6$ platelets per µl, which is 3 to 4 times baseline counts (Weibrich et al., 2004, Bone, 34(4):665-71).

In addition, the obtaining of platelet concentrates still needs the use of relatively complex kits and costly dedicated machinery and the equally costly involvement of specialized technicians. This drawback makes the current known methods of preparation of PRP inadapted to a point-of-care use.

Therefore, there is a need for new or alternative method of preparation of PRP suited for platelet separation from red blood cells and for platelet sequestration in high concentration while preserving their integrity, notably in terms of growth factors secretion ability and viability.

**Summary of the invention**

The invention relates to a new platelet-rich plasma preparation, a method of preparation of a new platelet-rich plasma, a use of such a platelet-rich plasma and preparations containing such a platelet-rich plasma preparation.

In a first aspect, the present invention provides a process for the preparation of a platelet concentrate composition, comprising the steps of:

a) Centrifuging whole blood in a separator tube selected from:
   - a glass separator tube containing a polyester-based thixotropic gel and a buffered sodium citrate solution at 0.10 M; and
- a polyethylene terephthalate separator tube containing a highly thixotropic gel formed by a polymer mixture and an anhydrous sodium citrate at 3.5 mg/mL;

b) Separating the enriched platelet rich plasma from the full plasma by removing half of the supernatant containing the platelet poor plasma;

c) Re-suspending the enriched plasma;

wherein the centrifugation step is performed at a force of or about 1500g up to about 2000g in a sufficient length of time to form a barrier between the plasma containing the platelets, the lymphocytes and the monocytes and the pellet containing the erythrocytes; the separation step b) is made by collecting the supernatant from atop of said barrier and wherein the enriched plasma is enriched in leucocytes, thrombocytes and adhesion proteins (for example, fibronectin) as compared to native whole blood.

In a second aspect, the present invention provides an isolated platelet concentrate composition comprising:

a) plasma;

b) platelets at a concentration of at least 30 Ox 10⁹ cells/L;

c) white blood cells at a concentration of at least 7.0 Ox 10⁹ cells/L;

d) fibrinogen at a concentration of at least 3 mg/L;

and wherein the erythrocyte concentration is less than 0.6 x 10¹² cells/L.

In a third aspect, the present invention provides a wound healant composition comprising:

a) plasma;

b) platelets at a concentration of at least 30 Ox 10⁹ cells/L;

c) white blood cells at a concentration of at least 7.0 Ox 10⁹ cells/L;

d) fibrinogen at a concentration of at least 3 mg/L;

e) coagulation activator in a vol. ratio (platelet concentrate: coagulation activator) of about 10:1 to about 10:3;

f) optionally admixing an autologous cell extract, such as an extract of keratynocytes, bone marrow, chondrocytes, fibroblasts, periosteam or corneal cells;

and wherein the erythrocyte concentration is less than 0.6 x 10¹² cells/L.

In a fourth aspect, the present invention provides a process for the preparation of a wound healant composition comprising:

a) Providing a platelet concentrate of the invention:
b) Admixing the platelet concentrate with a coagulation activator in a vol. ratio
   (platelet concentrate: coagulation activator) of about 10:1 up to about 10:3;

c) Optionally admixing with autologous cell extract, such as extract of
   keratynocytes, bone marrow, chondrocytes, fibroblasts, periosteum or corneal cells.

In a fifth aspect, the present invention provides a device for the preparation of a
platelet concentrate from whole blood comprising a separator tube wherein the
separator tube is selected from:
- a glass separator tube containing a polyester-based thixotropic gel and a buffered
  sodium citrate solution at 0.10 M; and
- a polyethylene terephthalate separator tube containing a highly thixotropic gel
  formed by a polymer mixture and an anhydrous sodium citrate at 3.5 mg/mL;
characterised in that the device has an inlet for introducing said whole blood, is held
in a vacuum intended to aspirate the whole blood sample, is sterile, has a usable
vacuum of or about 8 to about 10 mL and is suitable for undergoing centrifugation.

In a sixth aspect, the present invention provides a use of a platelet concentrate
according to the invention for the manufacture of a medicament for healing of wounds
or for promoting bone or periodontum growth and/or bone and/or tissue regeneration.

In a seventh aspect, the present invention provides a use of a platelet concentrate
according to the invention for the manufacture of a cosmetic preparation for use as
anti-aging agent or skin repairing agent such as a scar repairing agent, a wrinkle
filling and/or repairing agent.

In an eighth aspect, the present invention provides a pharmaceutical composition
comprising platelet concentrate according to the invention and a pharmaceutically
acceptable carrier.

In a ninth aspect, the present invention provides a cosmetic composition comprising
platelet concentrate according to the invention and a cosmetically acceptable carrier.

In a tenth aspect, the present invention provides an implantable device for use in
tissue regeneration therapy comprising:
  a) a permeable core comprising a platelet concentrate of the invention; and
b) an external jacket surrounding said core, said jacket comprising a biocompatible material, preferably bioresorbable.

In an eleventh aspect, the invention provides a kit adapted for tissue regeneration comprising a separator tube according to the invention, phlebotomy accessories for the preparation of the wound healant according to the invention and an applicator device (e.g. a double syringe) for the simultaneous dispensation onto the wound of the platelet concentrate according to the invention and a coagulation activator.

In a twelfth aspect, the invention provides a method for promoting wound healing and/or sealing and/or tissue and/or bone regeneration in a wound of a human or a lower animal comprising:

a) Providing a wound healant according to the invention;
b) Applying a therapeutically effective amount of the said wound healant to a wound, a damaged tissue or a damaged bone.

In a thirteenth aspect, the invention provides a method for inducing periodontal regeneration in a wound or a periodontal defect of a mammal with periodontal disease or other condition requiring periodontal regeneration comprising:

a) Providing a wound healant according to the invention;
b) Applying a therapeutically effective amount of the said wound healant to the said wound or said periodontal defect or cavity;
c) Optionally inserting a periodontal barrier, wherein the barrier is positioned between the gingival tissue and the wound treated according to steps a) and b) and the said barrier is selected from a membrane, a biodegradable polymer and/or a biocompatible porous material;
d) Closing the wound.

In a fourteenth aspect, the invention provides a method for promoting skin regeneration in a scar or a wrinkle from human or lower animal comprising:

a) Providing a wound healant according to the invention;
b) Filling the skin scar or wrinkle line with the said wound healant.

The methods, the devices and the kit according to the invention present the advantages to provide a time-effective and relatively low-cost way of obtaining platelet concentrates in a single operation that is easy to implement and adapted to a
point-of-care application. The methods of the invention present the advantage to not only lead to enriched preparations wherein the platelet are concentrated in such a high yield that is not obtained by known methods but also wherein the content in erythrocytes is much lower that those obtained by known methods for the preparation of PRP. The compositions of the invention presents the advantage of having a higher content in platelets, a lower content in erythrocytes than PRP obtained by known methods and completely maintained properties for its subsequent therapeutic use in-vivo. More specifically, the ability of the platelets to release the principal growth factors involved in tissue regeneration (PDGF, TGF-β, IGF, VEGF and EGF) at levels for several days (or the 7-10 day life span of thrombocytes) is maintained.

In addition, to the extent the compositions of the invention are made from autologous blood, the invention described herein reduces the disease transmission and immunoreaction risks associated with the use of the treatment materials made from biological materials obtained from one or more third parties.

**Description of the figures:**

**Figure 1** is a schematic representation of the variation of concentration in growth factors (PDGF-AB, EGF and VEGF) of a platelet concentrate composition according to the invention versus time (T in hours) after the centrifugation step in the preparation process of the invention.

**Figure 2** is a schematic representation of the outcome of the treatment of a skin graft donor site with a preparation containing a platelet concentrate composition according to the invention in comparison with a control group in terms of healing time in days (HT), pain at day 5 on a scale 0 to 10 (P) and epithalization at day 5 on a scale 0 to 7 (E). Control group: C, platelet-rich preparation alone: ReGenPRP™, platelet-rich preparation and autologous keratynocytes: ReGenExtracell™. The dotted line indicates when the first bandage is changed at day 5.

**Detailed description of the invention:**

The following paragraphs provide definitions of the terms according to the invention and are intended to apply uniformly throughout the specification and claims unless an otherwise expressly set out definition provides a broader definition.
By the expression "thixotropic" is meant a gel that becomes more fluid as a result of agitation or pressure, i.e. a gel which viscosity is decreasing as a result of agitation or pressure. The term viscosity refers to those characteristics of the specified material(s) determining the degree of gelation, such as for example the firmness or hardness of the material, the degree to which the material resists flowing like a fluid. A thixotropic gel according to the invention comprising a polyester gel or a mixture thereof which is water insoluble and chemically inert to blood constituents which can be used in accordance with the invention. Typical thixotropic gels are used in blood cells separation for diagnostics and proteomics purposes.

By the expression "point-of-care" is meant all services provided to patients at the bedside.

By the expression "phlebotomy accessories" or "venipuncture accessories" is meant accessories that allow the puncture of a vein with a needle for the purpose of drawing blood.

By the expression "wound healant" or "wound sealant" is meant an agent or a composition that is able to promote and/or increase the speed and/or quality of cicatrization of a wound. Wound healants or sealants are able to promote tissue regeneration.

By the expression "wound" is meant any damaged tissue, for example following trauma or surgery. Wounds in mammals, include for examples bed sores, ulcers, lacerations and burns, graft sites (graft donor and acceptor sites), fistulas, periodontal tissue damages, diabetic non-healing wounds, consequences of traumas or any surgery act. In its general sense the expression is intended to also encompass skin damages where the skin surface presents some depression without necessarily a cut on its surface such as age-related tissue damages (e.g. wrinkles) and scars such as for example acne or rubella scars.

By the expression "PRP" is intended to mean a platelet-rich-plasma, preferably of human origin, more preferably autologous, prepared by the process of the invention in order to pellet and remove erythrocytes and concentrate the plasma in leucocytes, thrombocytes and adhesion proteins as compared to native whole blood.

By the expression "autologous" or "autogenic" or "autogenous" is intended a method of the invention using a single donor's blood and wherein the blood extracted from this donor is intended for use on the same donor. As opposed, "allogenic" methods are
using blood from one or more third parties for use on a donor ("homologuous" or "heterologuous"). An autologous product avoids some of the common problems associated with the use of biological materials from third parties, such as for example screening to assure that the donor was biologically or immunologically compatible with the patient and potential contamination with hepatitis, HIV, prion, Creutzfeld-Jacob's disease and the like.

By the expression "coagulation activator" is intended an agent that is able to trigger or activate coagulation. A coagulation activator comprises a thrombin activator and/or a fibrinogen activator.

By the expression "thrombin activator" is intended an agent that is able to activate thrombin and to trigger coagulation. Typical thrombin activators are certain cofactors such as sodium or calcium. In practicing this invention, thrombin activation preferably occurs in the presence of calcium ions. Calcium ions are generally added to the platelet concentrate as a salt solution to provide a final concentration generally of or about 0.1 mg/mL of platelet concentrate. Suitable calcium salts include, without limitation, CaCO$_3$, and CaSO$_4$. A preferred calcium salt for use in the invention is calcium chloride (CaCl$_2$). CaCl$_2$ is available as calcium chloride injection, USP 10% (Regen Lab, Switzerland).

By the expression "fibrinogen activator" is intended an agent that is able to activate the conversion of fibrogen into fibrin and triggers the formation of the clot. Typical fibrinogen activators are thrombin or batroxobin. The term thrombin may include calcified thrombin, in particular, from or about 100 to about 10 units of thrombin per 1 mL of 10% of aqueous calcium chloride solution; it may include calcified bovine thrombin, allogeneic thrombin or recombinant human thrombin, preferably autologous thrombin. A fibrinogen activator can be an enriched thrombin composition such as thrombin compositions as described in US 6,472,162 or an autologous thrombin serum according to the invention.

By the expression "therapeutically effective amount" is intended the amount or amounts of the constituent elements or combination thereof necessary to enhance wound healing such as, for example, the reduction in the volume or surface area of a wound, the increase in the amount of granulation tissue or other biological material facilitating collagen lay down, vascular in growth, fibroblast proliferation or overall healing; All of the versions of the invention described herein are assumed to have the therapeutically effect amount(s) of constituent substances, or combinations thereof.
By the expression "pharmaceutically acceptable carrier" is intended pharmaceutically acceptable additional ingredient such as stabilizers, antimicrobial agents, buffers, adjuvants, anaesthetics, corticosteroids and the like.

By the expression "cosmetically acceptable carrier" is intended cosmetically acceptable additional ingredient such as stabilizers, buffers, colouring agents, flavouring agents, adjuvants, and the like.

The compositions according to the invention are particularly useful in wound healing treatments, especially the treatment of traumatic or surgical wounds such in the fitting and/or holding and/or sealing of native or prosthetic grafts (especially skin, bone grafts and dental prostheses or implants or the like, including also the graft donor site), treatment of vascularitis, ulcers, radiodermatitis (e.g. after irradiation on an epidermoidal skin carcinoma) and closing fistulas (such as for cyclists).

The use of the resulting platelet-rich plasma of the invention can be further modified before application and according to the therapeutic objective.

Compositions of the invention can be used together with bone filling materials, especially resorbable filling materials such as hydroxyapatite (calcium phosphate ceramic used as a biomaterial) or demineralised bone, or used as a mixture with bone extracts in a process for the regrowth of bone for example in craniofacial and orthopaedic procedures.

Compositions of the inventions may be used as a wound sealant in plastic surgery including burn grafting and other free skin graft applications, for example in oncology for favouring tissue regeneration, including speeding (neo)vascularization.

The compositions according to the invention are particularly useful in wound healing treatments at the skin graft donor site. The removal of a skin graft on a healthy skin creates a new wound at the donor's site which normally heals spontaneously between 12 to 14 days. However, this cicatrisation is extremely demanding for the body, especially if the donor site is broad or the person is less resistant (e.g. burn victims, people suffering from multiple traumas, people treated with corticoids, children or elderly) and the energetic losses are even increased by the loss in minerals, trace elements and proteins induced by the fluid losses from the new wound. In addition, important pain during the first 8 days is often present on the graft donor's site. Pain
reduction treatments are often used such as the use of analgesics (e.g. morphin) and/or hydrocellular wound dressings, however pain remains present, especially during the dressing change that occurs imperatively within 48 hours up to 1 week after the graft removal. In addition, the hydrocellular wound dressings have the drawbacks not only to be rather expensive but also by maintaining humidity on the wound, to prevent its drying, to increase the wound deepness, to favour the outbreak of bacterial infections and to lead to non-esthetic scars. Therefore, a stimulation of the skin graft donor site healing is very desirable.

Compositions of the invention are particularly adapted to chronic wounds that may lack sufficient blood circulation to facilitate the wound healing cascade.

The composition according to the invention may be also used in the treatment of periodontal disease where a loss and/or a damage of the periodontal tissues is observed, such a treatment comprising for example placing at the periodontal site or cavity in a human or a lower animal in need of periodontal tissue regeneration a composition according to the invention.

The compositions according to this invention are effective in eliminating or greatly reducing post-operative bleeding and extravasation or loss of serous or other fluid in these applications, in reducing the infection risk caused by most bacteria and/or enhances connective tissue formation compared to natural healing (i.e. no exogenous agents added) or to healing obtained through the use of other platelet concentrates prepared with known methods.

The compositions according to the invention are particularly useful in the preparation of pharmaceutical for promoting and/or initiating wound healing or for the preparation of cosmetic compositions for skin regeneration such as reducing skin wrinkles, acne or rubella scars, vitiligo and lipoathrophy (e.g. anti-aging compositions and skin regeneration compositions).

The compositions of the present invention may be administered locally or injected in the wound or subcutaneously. Local administration may be by injection at the site of injury or defect or by insertion or attachment of a solid carrier at the site, or by direct, topical application of the composition of the invention. Preferably, the compositions are readily syringable compositions.
The compositions of the present invention may be administered in combination with a co-agent useful in the treatment of tissue regeneration such as a healing agent, a wrinkle filler, an anti-aging agent or an antibacterial agent. The invention comprises compositions combined with a co-agent useful in the treatment of tissue regeneration for simultaneous, separate or sequential use in tissue regeneration therapy such as wound healing, bone and periodontum growth repair.

The compositions of the invention, the device and procedures for the preparation of autologous platelet concentrates for the invention are particularly useful for therapeutic use, particularly as autogenous biological glue in a haemostatic system intended to accelerate the physiological process of tissue regeneration, for example in dental implantology, skin and bone surgery. The compositions of the invention, the device and procedures for the preparation of autologous platelet concentrates for the invention are particularly useful for cosmetic use, particularly as autogenous rejuvenation material intended to be used for example as wrinkle or scar filler, alone or in combination with at least one anti-aging agent.

The platelet concentrate of the invention may be combined with an autologous cell extract preparation such as for example keratynocytes, bone marrow cells, chondrocytes, fibroblasts, periosteum or corneal cells. Keratynocytes can be harvested through a method described by Reinwald and Green, 1975, Cell, 6(3):331-43. Other mentioned cells can be harvested through methods described in "Culture de cellules animales; méthologies-applications", 2003, Ed. Barlovatz-Meimom and Adolphe, INSERM editions, Paris. Alternatively, cell extracts are derived from a cell bank or a cell culture.

The platelet concentrate of the invention has proven to be really beneficial in the acceleration and/or promotion of the healing process of wounds, even chronic unhealing wounds, leading to successful closures where weeks of conventional therapies had failed and achieving a decrease in infection risks, an improvement in patient’s recover and comfort, a reduction of medical care costs and a better esthetic final result.
The compositions of the invention can of course be also prepared from plasma derived from several identified donors. The invention is not limited to autologous biological materials, such as collection of concentrated platelets from the wounded's own biological material. The invention encompasses the use of biological materials obtained from one or more third parties, who need not be of the same species as the patient whose wound is being treated with the wound healant composition described herein unless bio-incompatibility would result from the use of such third party biological materials.

The following abbreviations refer respectively to the definitions below:

ATS (autologous thrombin serum); BU (Baxothrin unit); EC (Enriched clot); HT (healing time); IU (International Unit); PBS (Phosphate Buffered Saline); PET (polyethylene terephthalate); PRP (platelet-rich plasma); PPP (platelet-poor plasma); USP (United States Pharmacopeia); cm (centimeter); dL (deciliter); g (gram); Gy (gray); L (liter); min (minute); mm (millimeter); M (molar); mL (milliliter); rpm (Rotation per minute); Vol. (volume).

In one embodiment, the invention provides a process for the preparation of a platelet concentrate composition as described herein.

In a further embodiment, is provided by the invention a process for the preparation of a platelet concentrate composition wherein the centrifugation step is performed at force between about 1'500 g and up to about 1'700 g for a time selected from about 3 min up to about 15 min, preferentially at 1'500 g for about 8 min.

In another further embodiment, is provided by the invention a process for the preparation of a platelet concentrate composition wherein the separator tube has an inlet for introducing said whole blood, is held in a vacuum intended to aspirate the whole blood sample, is sterile, has a usable vacuum of 8 to 10 mL and is suitable for undergoing centrifugation.

In another further embodiment, is provided by the invention a process for the preparation of a platelet concentrate composition wherein the separator tube is a polyethylene terephthalate separator tube containing a highly thixotropic gel formed by a polymer mixture and an anhydrous sodium citrate at 3.5 mg/mL.
In another embodiment, the present invention provides an isolated platelet concentrate composition obtainable from the process according to the invention.

In another embodiment, is provided by the invention an isolated platelet concentrate composition comprising:

a) plasma;
b) platelets at a concentration of at least 300x10^9 cells/L, preferably of at least 350x10^9 cells/L, more preferably of at least 400x10^9 cells/L;
c) white blood cells at a concentration of at least 7.0x10^9 cells/L, preferably of at least 8.0x10^9 cells/L;
d) fibrinogen at a concentration of at least 3 mg/L;

and wherein the erythrocyte concentration is less than 0.5x10^12 cells/L, preferably less than 0.5x10^12 cells/L.

In a third aspect, the present invention provides a wound healant composition comprising:

a) plasma;
b) platelets at a concentration of at least 300x10^9 cells/L, preferably of at least 350x10^9 cells/L, more preferably of at least 400x10^9 cells/L;
c) white blood cells at a concentration of at least 7.0x10^9 cells/L, preferably of at least 8.0x10^9 cells/L;
d) fibrinogen at a concentration of at least 3 mg/L;
e) a coagulation activator in a vol. ratio (platelet concentrate: coagulation activator) of about 10:1 to about 10:3;
f) optionally an autologous cell extract, such as extract of keratynocytes, bone marrow, chondrocytes, fibroblasts, periosteum or corneal cells;

and wherein the erythrocyte concentration is less than 0.5x10^12 cells/L, preferably less than 0.5x10^12 cells/L.

In another embodiment, is provided by the invention a process for the preparation of a wound healant composition as described herein.

In another further embodiment, is provided by the invention a process for the preparation of a wound healant composition wherein the coagulation activator which is admixed is 10% calcium chloride.
In another further embodiment, is provided by the invention a process for the preparation of a wound healant composition wherein the coagulation activator which is admixed under step b) is a thrombin enriched preparation. A method for preparing thrombin for use in a biological glue is described in US 6,472,162 by the addition of 8 to 20% ETOH to a volume of plasma and this preparation may be used as a thrombin enriched preparation in the context of the invention. Alternatively, an autologous thrombin serum (ATS) can be used a thrombin enriched preparation in the context of the invention. An autologous thrombin serum according to the invention is obtained by a process comprising (i) the addition to a patient's whole blood sample (e.g. 8 mL) collected in a separator tube of the invention, a 10% of final volume of calcium chloride 10% (e.g. 1 mL) and a 10% of the final volume of a preparation of 95% v. ethanol solution (e.g. 1 mL) and (ii) precipitation for about 30 min at room temperature. After 30 min, a centrifugation at or about 1'500 g for about 8 to 10 min. In a further preferred embodiment, the thrombin enriched preparation and preferably the autologous thrombin serum is admixed under step b) directly on the wound.

In another further embodiment, is provided by the invention a process for the preparation of a wound healant composition wherein the cell extract which admixed at step c) is an autologous extract of keratynocytes.

In another further embodiment, is provided by the invention a process for the preparation of a wound healant composition according to the invention wherein a further step b') wherein the activated platelet-rich preparation composition (obtained by the admixing of the platelet concentrate with the said coagulation activator) obtained in step b) may be partially dehydrated by the contact of a wound dressing covered by a soft hydrophobic layer to avoid contamination with micro-strings from the dressing in order to obtain a semi-solid gel that can be manipulated by appropriate instruments, for example to fill a cavity or tissue deficiency, or as a growth matrix ("scaffold") while waiting for the reconstitution of the autogenous extracellular matrix. The obtained wound healant is particularly useful in a method for inducing periodontal regeneration in a wound or a periodontal defect or a cavity.

In another further embodiment, the isolated platelet concentrate composition, the wound healant composition, the thrombin enriched serum and/or the cell extract of the invention is/are autologous.
In another embodiment, the present invention provides a device for the preparation of a platelet concentrate composition from whole blood as described herein.

In another embodiment, the present invention provides a use of a platelet concentrate composition according to the invention for the manufacture of a cosmetic preparation for use as anti-aging agent or skin repairing agent such as scar repairing agent, wrinkle filling and/or repairing agent.

In another embodiment, the present invention provides a pharmaceutical composition comprising platelet concentrate according to the invention and a pharmaceutically acceptable carrier.

In another embodiment, the present invention provides a cosmetic composition comprising platelet concentrate according to the invention and a cosmetically acceptable carrier.

In another embodiment, the present invention provides an implantable device for use in tissue regeneration therapy as described herein.

In another embodiment, the present invention provides a kit adapted for tissue regeneration as described herein.

In a further aspect, the present invention provides a kit adapted for tissue regeneration according to the invention wherein the kit further comprises separate vials containing ETOH and CaCl₂, syringe holders, clumper and a tip applicator with a dual exit.

In a further aspect, present invention provides a kit adapted for tissue regeneration according to the invention comprising two sterile blisters:

1. one blister comprising accessories for the phlebothomy, separator tubes of the invention, vials of ETOH and CaCl₂ for the preparation of an autologous thrombin serum; and
2. a second blister comprising accessories for two syringe holders and clumper, and tip applicator with a dual exit.
In another embodiment, the present invention provides a method for promoting wound sealing and/or tissue and/or bone regeneration in a wound of a human or a lower animal as described herein.

In another further embodiment, the present invention provides a method for promoting wound sealing and/or tissue and/or bone regeneration in a wound of a mammal, preferably human.

In another embodiment, the present invention provides a method for inducing periodontal regeneration in a wound or a periodontal defect of a mammal with periodontal disease or other condition as described herein.

In another further embodiment, the present invention provides a method for inducing periodontal regeneration in a wound or a periodontal defect or cavity of a mammal with periodontal disease or other condition wherein the mammal is human.

In another further embodiment, the present invention provides a method for inducing periodontal regeneration in a wound or a periodontal defect or cavity according to the invention wherein the said therapeutically effective amount of the said wound healant composition is applied in a form of semi-solid gel or a growth matrix to the said wound or said periodontal defect or cavity such as described for example in Garg et al, 2000, Dental Implantology Update, 11(6), 41-44.

In another embodiment, the present invention provides a method for promoting skin tissue regeneration in a scar or wrinkle as described herein.

Said method of treating a wound may include the use of any of the compositions described herein; it may also include the use of any composition made by any of the methods described herein.

Examples illustrating the invention will be described hereinafter in a more detailed manner and by reference to the embodiments represented in the Figures.

The invention therefore provides an improved biological wound healing material, preferably autologuous, promoting tissue, skin and bone regeneration, especially cicatrisation. The benefits of the invention comprise a simple and rapid method of preparation of improved wound healing and tissue regenerating materials adapted to
point-of-care services and which proved to decrease the healing time, associated pain and medical costs. Further, the improved wound healing and tissue regenerating materials lead to scars having a much better aesthetic final aspect and to the durable filling of scars and wrinkles.

EXAMPLES

GENERAL PROCEDURES & CONDITIONS
To determine the effectiveness of compositions of the invention in promoting wound healing and/or bone and/or tissue regeneration, the following experiments are performed.

Whole human blood sample is collected in a separator tube according to the invention. A separator tube according to the invention is for example an approximately 15 mL glass tube (16 mm diameter and 130 mm in length) containing 3 mL of polyester-based thixothropic gel as well as 1 mL of sodium citrate solution at 0.1 M and containing a usable vacuum of or about 8.5 mL. This separator tube constitutes a ready-to-use device for the preparation of a platelet concentrate composition of the invention (also called RegenTHT™ (Thrombocyte Harvesting Tube) from Regen Lab, Switzerland).

Another example of a separator tube according to the invention is a tube of approximately 10 mL in PET (polyethylene terephthalate) containing 1 mL of a thixotropic gel comprising a polymer mixture and anhydrous sodium citrate deposited on the inner surface of the tube by spraying (about 3.5 mg per mL of blood) and containing an usable vacuum of or about 8 mL, constitutes a ready-to-use device for the preparation of a platelet concentrate according to the invention (also called RegenBCT™ (Blood Cell Therapy) from Regen Lab, Switzerland).

These tubes are sterilized by irradiation (such as prescribed by ISO 11137, UNI EN ISO 11737-2, UNI EN 552, UNI EN 556) and hermetically sealed by a traditional cap such mottled bromobutyl conventional rubber stopper for the glass tube and a chlorobutyle stopper having a polyethylene cover for the operator safety.

Then, the separator tube is centrifuged at or about 1'500g up to or about 2'000g for about 3 to 10 min, i.e. of or about 2'500 rpm up to or about 3'800 rpm with a centrifuge with a swinging rotor, having a radius of 20 cm. In case of a centrifuge
having a rotor with a fixed angle of about 45°, the centrifugation time should last for at least about 15 min.

After centrifugation, the platelet concentrate is collected for use in therapeutic or cosmetic applications of the invention or for the preparation of further compositions containing the obtained platelet concentrate through the mixture with further agents such as cell extracts, preferably autologous (e.g. keratynocytes, fibroblasts, bone marrow cells, chondrocytes or corneal cells) and/or bone substitutes and/or coagulation activators.

For the therapeutic applications, a kit according to the invention adapted for tissue regeneration is used wherein the kit (also called RegenKit™) comprises two sterile blisters comprising:
- one blister (RegenPRP™) comprising accessories for the phlebothomy, separator tubes of the invention (ReGenTHT™ or ReGenBCT™), vials of ETOH and CaCl₂ for the preparation of an autologous thrombin serum (ReGenATS™).
- one blister (RegenApplicator™) comprising two syringes (e.g. ImI and 1 or 3 mL), holders and clumper and a tip applicator with a dual exit.

**Example 1: Preparation of an autologous platelet concentrate**

Separator tubes of the invention are beforehand tested for the good tolerability, the non-toxicity and the non-mutagenicity of the thixothropic gel according to norms ISO 10993-11, ISO 10993-10, ISO 10993-12 and ISO 10993-3.

About 8.5 to about 10-mL of human blood sample are collected within the separator tube of the invention, where the blood is aspirated by the vacuum. The mixture is then centrifuged at approximately 3’800 rpm for about 3.5 min. The platelet-rich plasma is then collected.

The analysis of the platelet concentrate obtained by the method of the invention has shown that it contains 2 to 4 times the normal levels of platelets and growth factors, compared to a natural blood clot, while maintaining normal levels of fibrin and fibrinogen and containing practically no blood cells (< 1% hematocrit, compared to 35-50% in a normal blood clot and 15-20% in platelet-rich plasma obtained from known methods of preparation). The study shows also the presence of leukocytes glycoprotein fibronecton and this demonstrates that the coagulating properties are preserved.
The composition of the platelet concentrate (also called RegenPRP™ from Regen Lab, Switzerland) compared to whole blood, whole plasma and platelet-poor plasma is presented in table 1 below:

Table 1

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<th>Sample</th>
<th>White Blood Cells (x10^9/L)</th>
<th>Red Blood Cells (x10^12/L)</th>
<th>Hemoglobin (g/dL)</th>
<th>Platelets (x10^9/L)</th>
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<tr>
<td>Complete blood</td>
<td>6.6</td>
<td>4.43</td>
<td>13.5</td>
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<td>Complete plasma</td>
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<td>Platelet-poor plasma</td>
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<td>0.1</td>
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</table>

The experiment was repeated on several patients and it was observed that the obtained platelet concentrates present reproducible concentrations for platelets (at least 30x10^9 cells/L), white blood cells (at least 7.0x10^9 cells/L), fibrinogen (at least 3 mg/L) and erythrocytes (less than 0.6x10^12 cells/L).

The platelet yield obtained by such the method of the invention has been measured (90-99%) and shows to be drastically increased in comparison with the platelet yields (30-62%) obtained from known methods of preparation described in Marx et al, 2004, above.

In addition, it has been shown through ELISA kits (R&D Systems, Inc.) and the response to coagulation activation of the platelet concentrate of the invention, that the activity of coagulation factors is preserved: the concentration of D-dimers (fibrin breakdown products), known markers of coagulation activation, and the lysis process are stable and therefore the coagulation properties of the platelet concentrate are not weakened by the process of the invention.

The levels of growth factors (PDGF, EGF, TGF-b and VEGF) from the platelet concentrate of the invention are demonstrably stable for a period of at least 72 hours (4 days) when stored at room temperature in the sterile separator tube of the invention. The evolution of growth factors PDGF BB, EGF and VEGF over 72 hours is presented on Figure 1.

The properties of the platelet concentrate according to the invention make it possible to envisage preparing platelet concentrate obtained using the invention's procedure, one to several days before a reparative surgery, in order to reduce the workload in the operating room and speed-up the surgical procedure.
For subsequent therapeutic use, the autologous platelet concentrate is generally mixed with a conventional coagulation activator such as a thrombin activator (e.g. calcium chloride pour example at 10%), optionally mixed with a fibrinogen activator such as thrombin, preferably homologous (e.g. 10 UI to 100 IU per mL of plasma), batroxobin (e.g. 20 BU per mL of plasma) or a thrombin enriched preparation.

**Example 2: Therapeutic use of the autologous platelet concentrate of the invention**

**a) Patients:**

Three patients presenting chronic unhealing wounds are selected:

- One 88-year-old patient (Patient 1) suffering from multiple locations Karposi’s angiosarcoma on lower limbs and from a radio-induced necrosis on the left leg. The radio-induced necrosis was resulting from a radiotherapy treatment. After 12 months after the end of the low-voltage X-ray treatment, the necrosis was consisting in a deep surinfected ulcer surrounded by a scrab (35x25 mm). The wound had been previously unsuccessfully treated with various treatments such as with steroids and healing creams.

- One 81-year-old patient (Patient 2) suffering from a vertex spinocellular carcinoma was presenting a cutaneous ulceration (about 10 mmm diameter) with peripheral dyskeratosis without any infection sign resulting from a biopsy-resection and a post-surgical radiotherapy (total dose of 52 Gy).

- One 60-year-old patient (Patient 3) having received a pre-surgical irradiation (7 Gy) for tibia and fibula synostosis on the right leg was presenting a radio-induced necrosis consisting in a deep ulcer (50x30 mm diameter) without inflammation.

**b) Treatment:**

8.5 mL of blood sample is taken from each patient and centrifuged in a separator tube as described in Example 1, according to the protocol as described in Example 2. The resulting platelet concentrates are then mixed with calcium chloride at 10% vol.. Each autologous platelet concentrate composition is then applied on the radio epidermitis wound site of the corresponding patient. The wound is then covered and protected with humid compresses (Day 1).

Between days 3 and 5, the wound status is checked and the wound dressing is changed. At day 7 ±1, a new application of a new autologous platelet concentrate
preparation of the invention is performed. If needed, the same treatment sequence is followed with the same time intervals till the complete cicatrisation of the wound.

c) **Healing** effects:

Patient 1: Slow and regular healing of the ulcer. Complete cicatrisation after 189 days.

Patient 2: Very quick healing obtained in 21 days.

Patient 3: Progressive and regular healing. Complete cicatrisation after 41 days.

These results show the benefit effect of the platelet concentrate composition of the invention in the healing of chronic radio-induced ulcers, even in the case of those which were resistant to previous topic treatments and in the absence of any allergic reaction.

**Example 3: Therapeutic use of the autologous platelet concentrate of the invention in combination with an autologous thrombin enriched serum**

To activate coagulation, an alternative to the mixture of the platelet concentrate of the invention with a thrombin activator before the use on a patient, as described in Example 1, is the combination of the platelet concentrate of the invention with a fibrinogen activator such as a thrombin enriched composition and preferably with a thrombin serum (e.g. autologous) according to the invention.

a) **Preparation of an autologous thrombin serum (ATS)**

An autologous thrombin serum to be used as a thrombin enriched preparation in the context of the invention is prepared by a process which comprises the addition to a patient's whole blood sample (e.g. 8 mL) collected in a separator tube of the invention as described in Example 1, a 95% v. ethanol solution (e.g. 1 mL) and calcium chloride 10% (e.g. 1 mL). The mixture (also called RegenATS™ from Regen Lab, Switzerland) is then allowed to precipitate for about 30 min at room temperature. After 30 min, almost 80% of the anti-thrombin (among other proteins like fibrinogen) is precipitated; then the tube is centrifuged at or about 1500 g for about 8 to 10 min and the autologous thrombin serum is ready for use in combination with the platelet-rich concentrate of the invention.

b) **Combined preparations**

One of the originality of this process is that after the initial step of incubation of the autologous thrombin serum preparation process (e.g. at least about 30 min), the separator tubes of the invention containing respectively the autologous thrombin serum preparation and the platelet concentrate preparation can be centrifuged...
simultaneously in order to get the two blood extract preparations ready for use at the same time.

c) Combined use
To allow the polymerization of fibrinogen into a fibrin mesh (which occurs during the coagulation process) to occur only at the moment of application of the platelet-rich preparation on the wound, the platelet concentrate composition and autologous thrombin serum (coagulation activator) are applied simultaneously at a vol. ratio of about 10:1 to about 10:3 (concentrate to coagulation activator ratio) to the wound.

The simultaneous delivery of both preparations is achieved for example by a device comprising two syringes (e.g. 10-mL syringe for the platelet concentrate composition and a 1-mL or 3-mL syringe for the thrombin serum), that releases the preparations simultaneously so that they mix and polymerize upon contact with the wound.

Example 4: Therapeutic use of the autologous platelet concentrate of the invention in combination with cell extract
A total of 35 patients having received a skin graft (representing less than 15% of the skin surface) have been included in the study. Patients treated with immunosuppressants or corticoids or with renal insufficiency or severe peripheral artheropathy were excluded.

All the following manipulations are performed under the strict rules of asepsy and sterility.

Group A: 13 patients

a) Preparation of platelet concentrate
A 8.5 mL sample of whole blood from each patient (from a higher limb where no perfusion is present) is collected in a separator tube according to the invention. The separator tube with the whole blood is immediately centrifuged during about 8 min at 2'800 rpm. Before the enriched plasma (PRP) is collected, the operator discards the half or 2mL of the supernatant and then re-suspends the platelets in the remaining plasma. The platelet-rich concentrate is then transferred to a sterile tube maintained at a temperature of 37°C.

b) Wound coating
The autologous platelet concentrate of the invention (also called ReGenPRPTM) is mixed with a solution of calcium chloride 10% in a ratio 10:1 and the graft donor site
(where skin was removed) of each corresponding patient is coated with the autologous corresponding mixture in order to obtain coagulation of the platelet concentrate on the wound.

**Group B:** 8 patients

a) **Skin cell sampling on the patient**

Keratynocytes are extracted from each of the patients from this group. A thin healthy skin sample (about 2 cm²) is removed from each patient and washed three times in a PBS solution. The washed biopsy is then deposited in a Petri dish containing trypsin and cut into very small fragments (0.5 cm*0.5 cm) with a scalpel. The skin fragments are then incubated during 45 min at 37°C on a stirring device. The supernatant is then collected, centrifuged and cells are re-suspended in a PBS solution. The keratynocytes count is determined under microscope. Finally, the obtained keratynocytes were re-suspended in the autologous platelet concentrate from the corresponding patient.

b) **Preparation of platelet concentrate**

The procedure is the same as for Group A.

c) **Wound coating**

The keratynocyte suspension (also called ReGenExtractcell™) is applied as soon as ready (the entire preparation not exceeding a day) on the wound on the same way as described in the case of Group A.

**Control Group:** 14 patients

The graft donor site of each patient of this group is coated with a non-therapeutic compress (Jelonet®).

**Randomization and treatment**

In the surgery bloc, after the graft skin removal, the donor site is coated with a temporary compress soaked with an adrenaline solution (1 ampoule of 1mg/mL of adrenaline diluted in 500 mL NaCl 0.9%) and depending on the randomization table, the donor site is treated according to the three following methods:

Groups 1 and 2: Coating of the wound with the respective wound healing composition and covering of the wound with a non-therapeutic compress (Jelonet®).

Group 3: Direct covering of the wound with a non-therapeutic compress (Jelonet®).

The compresses are then covered with Kerlix® bands and elastic bands such as "Velpeau".
**Treatment efficacy criteria**

The efficacy of the treatment is evaluated according to 3 criteria:

- The time needed for the complete cicatrisation of the treated site (healing time or HT in days)

  - The epithelization (evolution of the cicatrisation progress) measured at day 5 after the treatment according to 7 degrees:

    0 : Absent
    1 : Slight
    2 : Moderate
    3 : Important
    4-7: Very important, increasing degrees of importance;

- The pain evaluated at day 5 after the treatment by the patient him/herself, generally at the time of compress change on a scale from 0 to 10 (0: no pain and 10: extreme pain).

The compress is opened at day 5 post-surgery to allow the evaluation of the quality of the treatment and covered with new Jelonet® compresses covered with dry compresses.

The compress is then changed very two days till the complete cicatrisation. Any side effects or medical complications are watched during the whole duration of the cicatrisation process.

**Results**

The results of the treatments for each patient group (Control group: C, Group A: ReGenPRP™, Group B: ReGenExtracell™) are presented on Figure 2 in terms of healing time in days (HT), pain at day 5 (P) and epithelization at day 5 (E). The dotted line indicates when the first bandage is changed at day 5.

The cicatrisation process is clearly stimulated by the use of the platelet concentrate of the invention as compared to the control group. The quality of the cicatrisation is also better in the case of the use of platelet concentrate of the invention. In addition, the pain at the donor site is dramatically reduced in the case where the platelet concentrate of the invention was used as compared to the control group.

All the beneficial effects of the platelet concentrate of the invention are increased when a mixture of keratynocytes suspended in the platelet concentrate of the invention is used.
The mean healing time is of 7 days for the group treated with a platelet concentrate of the invention and 5 days when keratynocytes are suspended in the platelet concentrate as compared to an average of 12 days in the control group.

Tolerability was excellent and no side effect or allergy has been detected.

This shows that the platelet concentrate of the invention alone or combined with keratynocytes is very efficient in accelerating the wound healing process and not only decreases the pain, but also the inflammatory reaction and improves the final aspect of the scar.

**Example 5: Cosmetic use of the autologous platelet concentrate of the invention**

An autologous platelet concentrate composition is prepared as described in Example 1. 5 mL of this platelet concentrate composition (also called RegenACR: (Autologous Cell Rejuvenation) from RegenLab, Switzerland) is injected subcutaneously in a wrinkle groove as wrinkle filling material, in the same way as commonly done with other wrinkle filler such as hyaluronic acid. The deepness of the wrinkle is progressively decreasing within the first weeks after the treatment and at the site of injection, a very clear regeneration of the area is obtained with an optimal result at two to three months. As opposed to what observed with other wrinkle filling materials, neither inflammation, nor swelling is observed at the site of injection and the benefit is durable as opposed to hyaluronic acid which is bio-resorbed after 4 to 6 months.

Claims

1. A process for the preparation of a platelet concentrate composition, comprising the steps of:
   a) Centrifuging whole blood in a separator tube selected from:
   - a glass separator tube containing a polyester-based thixotropic gel and a buffered sodium citrate solution at 0.10 M; and
   - a polyethylene terephthalate separator tube containing a highly thixotropic gel formed by a polymer mixture and an anhydrous sodium citrate at 3.5 mg/mL;
   b) Separating the enriched platelet rich plasma from the full plasma by removing half of the supernatant containing the platelet poor plasma;
   c) Re-suspending the enriched plasma;
wherein the centrifugation step is performed at a force of 1500g up to 2000g in a sufficient length of time to form a barrier between the plasma containing the platelets, the lymphocytes and the monocytes and the pellet containing the erythrocytes; the separation step b) is made by collecting the supernatant from atop of said barrier and wherein the enriched plasma is enriched in leucocytes, thrombocytes and adhesion proteins as compared to native whole blood.

2. A process according to claim 1 wherein the centrifugation step a) is performed at a force of 1'500 g up to 1'700 g for a time selected from 3 min up to 15 min.

3. A process according to claim 1 or 2 wherein the centrifugation step a) is performed at a force of 1'500 g for 8 min.

4. A process according to any one of claims 1 to 3 wherein the separator tube has an inlet for introducing said whole blood, is held in a vacuum intended to aspirate the whole blood sample, is sterile, has a usable vacuum of 8 to 10 mL and is suitable for undergoing centrifugation.

5. A process according to any one of claims 1 to 4 wherein the separator tube is a polyethylene terephthalate separator tube containing a highly thixotropic gel formed by a polymer mixture and an anhydrous sodium citrate at 3.5 mg/mL.
6. An isolated platelet concentrate composition comprising:
   a) plasma;
   b) platelets at a concentration of at least 30×10^9 cells/L;
   c) white blood cells at a concentration of at least 7×10^9 cells/L;
   d) fibrinogen at a concentration of at least 3 mg/L;
   and wherein the erythrocyte concentration is less than 0.6×10^12 cells/L.

7. A wound healant composition comprising:
   a) plasma;
   b) a platelet concentrate composition according to claim 6;
   c) a coagulation activator in a vol. ratio (platelet concentrate: coagulation activator) of 10:1 to 10:3;
   d) optionally an autologous cell extract, such as extract of keratynocytes, bone marrow, chondrocytes, fibroblasts, periosteum or corneal cells.

8. A wound healant composition according to claim 7 wherein the coagulation activator and/or the cell extract respectively is/are autologous.

9. A process for the preparation of a wound healant composition comprising:
   a) Providing a platelet concentrate composition according to claim 6;
   b) Admixing the platelet concentrate composition with a coagulation activator in a vol. ratio (platelet concentrate: coagulation activator) of 10:1 to 10:3;
   c) Optionally admixing with autologous cell extract, selected from keratynocytes, bone marrow, chondrocytes, fibroblasts, periosteum and corneal cell extract.

10. A process according to claim 9 wherein the coagulation activator which is admixed under step b) is 10% calcium chloride.

11. A process according to claim 9 wherein the coagulation activator which is admixed under step b) is an autologous thrombin enriched serum.

12. A process according to any of claims 9 to 11 wherein the cell extract which admixed at step c) is an autologous extract of keratynocytes.
13. A device for the preparation of a platelet concentrate from whole blood comprising a separator tube wherein the separator tube is selected from:

- a glass separator tube containing a polyester-based thixotropic gel and a buffered sodium citrate solution at 0.10 M; and
- a polyethylene terephthalate separator tube containing a highly thixotropic gel formed by a polymer mixture and an anhydrous sodium citrate at 3.5 mg/mL; characterised in that the device has an inlet for introducing said whole blood, is held in a vacuum intended to aspirate the whole blood sample, is sterile, has a usable vacuum of 8 to 10 mL and is suitable for undergoing centrifugation.

14. A kit adapted for tissue regeneration comprising device according to claim 13, phlebotomy accessories and an applicator device for dispensation simultaneously onto the wound of a platelet concentrate according to claim 6 and a coagulation activator.

15. Use of a platelet concentrate composition according to claim 6 or a wound healant composition according to claims 7 to 8 for the manufacture of a medicament for healing wounds or for promoting bone or periodontum growth or bone or tissue regeneration.

16. Use of a platelet concentrate composition according to claim 6 or a wound healant composition according to claims 7 to 8 for the manufacture of a cosmetic preparation for use as anti-aging agent or skin repairing agent such as scar repairing agent, wrinkle filling and/or repairing agent.

17. A pharmaceutical composition comprising a platelet concentrate composition according to claim 6 or a wound healant according to claims 7 to 8 and a pharmaceutically acceptable carrier.

18. A cosmetic composition comprising a platelet concentrate composition according to claim 6 or a wound healant according to claims 7 to 8 and a cosmetically acceptable carrier.
Figure 1
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61M1/36 B01D21/26 B01L3/14 G01N33/49

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)

A61M B01D B01L G01N A61B

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<td>US 5 667 963 A (SMITH WARD C [US] ET AL) 16 September 1997 (1997-09-16) column 8, lines 17-27 column 8, line 66 - column 9, line 22; figure 1 column 10, lines 49-55</td>
<td>13,14</td>
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Further documents are listed in the continuation of Box C

See patent family annex

**Date of the actual completion of the international search**

7 May 2007

**Date of mailing of the international search report**

21/08/2007

Name and mailing address of the ISA/European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Tx 31651 epo nl, Fax (+31-70) 340-3016

Authorized officer

Böttcher, Stephanie

Form PCT/ISA/210 (second sheet) (April 2005)
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</table>
Box II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. Claims Nos because they relate to subject matter not required to be searched by this Authority, namely

2. Claims Nos 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

3. Claims Nos because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

Box 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:

see additional sheet

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 an additional fee, this Authority did not invite payment of any additional fee

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 claims 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 claims Nos

see additional sheet(s)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest

☐ No protest accompanied the payment of additional search fees

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5,13,14

A process and a device for the preparation of a platelet concentrate composition comprising a separator tube containing a thixotropic gel and sodium citrate. Problem to be solved: Providing highly concentrated platelet-rich plasma while preserving the integrity of the platelets.

2. claims: 6-12,15-18

A platelet concentrate composition comprising plasma, platelets, white blood cells, fibrinogen and almost no erythrocytes, and the use of this concentrate for the preparation of a wound healant or a skin repairing agent. Problem to be solved: Enhance wound healing or skin repairing.
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<td>US 5667963</td>
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