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(54) **Title:** A COAGULATION MOLD, A KIT AND A METHOD FOR PREPARING A COAGULATED BLOOD MASS

(57) **Abstract:** The present disclosure provides a method, a coagulation mold and a kit for preparing a coagulated blood mass having a desired shape and volume. The coagulated blood mass is prepared from whole blood being withdrawn from a subject. The whole blood is mixed with coagulation initiators, i.e. coagulation agents or anti-anti coagulation agents, in a specific amount and having specific characteristics in order to obtain the coagulated blood mass with optimal parameters, such as stability, moisture, homogeneousness, namely the texture of the clot is substantially similar at any portion thereof, etc. This is obtained, inter alia, by selecting particle size of one of the coagulation initiators such as to yield more rapid dissolving within the blood and/or to yield improved suspension of the particles in the blood when it is still in a liquid form. Another parameter to yield the desired coagulated blood mass of the present invention is the selected amount of material of coagulation initiator to be mixed with the whole blood. By controlling theses parameters, an optimal coagulated blood mass is obtained that can be used for treatment of the human body, e.g. for treating skin lesions, internal injuries, such as anal fistula, stoma cavity, damaged tendon, and others.

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A COAGULATION MOLD, A KIT AND A METHOD FOR PREPARING A COAGULATED BLOOD MASS

TECHNOLOGICAL FIELD

The present disclosure is in the field of preparation of medical treatment, in particular preparation of a coagulated blood mass for use in a treatment of a subject.

BACKGROUND ART

References considered to be relevant as background to the presently disclosed subject matter are listed below:

- US 9,180,142
- US 2020/0281775
- WO 2019/150355

Acknowledgement of the above references herein is not to be inferred as meaning that these are in any way relevant to the patentability of the presently disclosed subject matter.

GENERAL DESCRIPTION

The present disclosure provides a method, a coagulation mold and a kit for preparing a coagulated blood mass, namely blood clot having a desired shape and volume. The coagulated blood mass is prepared from whole blood being withdrawn from a subject. The whole blood is mixed with coagulation initiators, i.e. coagulation agents or anti-anti coagulation agents, in a specific amount and having specific characteristics in order to obtain the coagulated blood mass with optimal parameters, such as stability, moisture, homogeneousness, namely the texture of the clot is substantially similar at any portion thereof, etc. This is obtained, *inter alia*, by selecting particle size of one of the coagulation initiators such as to yield more rapid dissolving within the blood and/or to yield improved suspension of the particles in the blood when it is still in a liquid form. Another parameter to yield the desired coagulated blood mass of the present invention is the selected amount of material of coagulation initiator to be mixed with the whole blood. By controlling

theses parameters, an optimal coagulated blood mass is obtained that can be used for treatment of the human body, e.g. for treating skin lesions, internal injuries (such as anal fistula, stoma cavity, damaged tendon, etc.).

Thus, a first aspect of the present disclosure provides a method for preparing a coagulated blood mass, i.e. a volumetric blood clot, for use in a treatment of a lesion or injury of a subject. The treatment can be on the external portion of the body such as skin lesions or on internal portions of the body such as fistula cavity, abdominal cavity, etc. The method comprising: mixing an amount of whole blood with calcium gluconate powder and kaolin powder; and allowing the whole blood to coagulate to form the coagulated blood mass. The term "allowing" defines that the mixture of blood with maintaining the mixture of whole blood with calcium gluconate powder and kaolin powder is maintained for a selected amount of time until the blood solidifies to a certain degree and seize to be in a flowable form. The amount of time can be more than 1 minute, 2, 3, 4, 5, 6, 7, 8, 9, 10 or even more than 15 minutes until the whole blood is solidified to the desired degree. The method is further characterized by at least one of: (i) the particle-size distribution D50 of the calcium gluconate powder is between 50μ-200μ, (ii) wherein said mixing comprises mixing between 0.6 mg – 3.1 mg of kaolin powder with every 1 ml of whole blood, (iii) wherein said mixing comprises mixing between 2.5 mg – 8.1 mg of calcium gluconate powder with every 1 ml of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 20μ.

The particle-size distribution DX means that X% of the particles in the powder are less than the nominal size associated with said distribution. For example, if the particle-size distribution D50 is 125μ then 50% of the particles are having a volumetric size equal or less to 125μ. By maintaining only particles with size that is relatively small, the resulted coagulated blood mass is homogenous, namely the volumetric solidity of the coagulated blood mass is substantially equal. This is resulted due to the duration that particles with such a size can suspend in the whole blood they are mixed with. Larger particles tend to sink in the blood and the resulted coagulated blood mass is not homogeneous.

It is to be noted that any combination of the described embodiments with respect to any aspect of this present disclosure is applicable. In other words, any aspect of the present disclosure can be defined by any combination of the described embodiments.

In some embodiments of the method, the particle-size distribution D70 of the calcium gluconate powder is between 50μ-200μ.

In order to prepare a coagulated blood mass with properties that are suitable for using in a medical treatment the calcium gluconate powder is first milled to a size of 2μ. The particles product of this milling is typically undergoing a certain degree of granulation, thus prior to the use of the powder in the formation of the coagulated blood mass, the calcium gluconate powder is sifted through a sifter of 120-125μ particles. Particle size of less than 120-125μ dissolve much faster and therefore the use thereof in the formation of a coagulated blood mass results in a much more stable and non-leaky coagulated blood mass.

In some embodiments of the method, particle-size distribution D90 of the calcium gluconate powder is between 50μ-200μ.

In some embodiments of the method, the particle-size distribution D90, D80, D70, D60, or D50 of the calcium gluconate powder is about 125μ.

The term "about" should be understood as a deviation of up to 20% from the nominal value. For example, if the nominal value is about 125μ, then the value should be considered as 100μ-150μ.

In some embodiments of the method, the particle-size distribution of either D50, D60, D70, D80, D90 or D95 of the calcium gluconate powder is between either 50μ-200μ, 60μ-190μ, 70μ-180μ, 80μ-170μ, 90μ-160μ, 100μ-150μ, 110μ-140μ or 120μ-130μ.

In some embodiments of the method, the particle-size distribution D99 of the kaolin powder is less than 50μ.

In some embodiments of the method, the particle-size distribution D99 of the kaolin powder is less than 50μ.

By using small particles of kaolin in the preparation of the coagulated blood mass, the kaolin particles suspend for a longer duration in the formed coagulating blood mass (when at least some of the blood is in liquid flowable form) and do not sink rapidly to the bottom of the formed coagulating blood mass, therefore resulting in a substantive homogeneous coagulated blood mass.

In some embodiments of the method, the particle-size distribution D80 of the kaolin powder is less than 25μ.

In some embodiments of the method, the particle-size distribution D90 of the kaolin powder is less than 3μ or between 1μ-3μ.

In some embodiments of the method, the particle-size distribution of either D50, D60, D70, D80, D90 or D95 of the calcium gluconate powder is less than either 30μ, 25μ, 20μ,15μ, 10μ, 5μ, 3μ, 2μ, 1μ or less than 0.1μ.

In some embodiments, the method comprising withdrawing said whole blood from the subject, therefore the coagulated blood mass is prepared from autologous whole blood.

In some embodiments, the method comprising, prior to said mixing, introducing anti-coagulant-containing liquid to said amount of whole blood. Namely, after the whole blood is withdrawn, it is added with anti-coagulant to prevent undesired coagulation of the blood.

In some embodiments of the method, said introducing comprises introducing between 0.04 ml – 0.095 ml of anti-coagulant-containing liquid for every 1 ml of whole blood.

In some embodiments of the method, said introducing comprises introducing about 0.07 ml of anti-coagulant-containing liquid for every 1 ml of whole blood.

In some embodiments of the method, said mixing is performed within a coagulation mold. The coagulation mold has a desired shape that determines the shape of the blood clot being prepared by the method.

In some embodiments of the method, the mixing comprises mixing between 0.6 mg – 3.1 mg of kaolin powder with every 1 ml of whole blood.

In some embodiments of the method, said mixing comprises mixing about 1.75 mg of kaolin powder with every 1 ml of whole blood.

In some embodiments of the method, said mixing comprises mixing between 0.8 mg – 2.9 mg, 1 mg – 2.7 mg, 1.2 mg – 2.5 mg, 1.4 mg – 2.3 mg, 1.6 mg – 2.1 mg or 1.8 mg – 2 mg of kaolin powder with every 1 ml of whole blood.

In some embodiments of the method, said mixing comprises mixing between 2.5 mg – 8.1 mg of calcium gluconate powder with every 1 ml of whole blood.

In some embodiments of the method, said mixing comprises mixing about 5.3 mg of calcium gluconate powder with every 1 ml of whole blood.

In some embodiments of the method, said mixing comprises mixing between 2.9 mg – 7.7 mg, 3.3 mg – 7.3 mg, 3.7 mg – 6.9 mg, 4.1 mg – 6.5 mg, 4.5 mg – 6.1 mg or 4.9 mg – 5.7 mg of calcium gluconate powder with every 1 ml of whole blood.

Yet another aspect of the present disclosure provides a coagulation mold comprising a coagulation space defined by a base surrounded by upwardly rising walls and configured to receive a selected amount of whole blood. The coagulation space comprises calcium gluconate powder and kaolin powder. The coagulation mold comprises at least one of the following: (i) the particle-size distribution D50 of the calcium gluconate powder between 50μ-200μ, (ii) kaolin powder in an amount of between 0.6 mg – 3.1 mg for every 1 ml of said selected amount of whole blood, (iii) calcium gluconate in an amount of between 2.5 mg – 8.1 mg for every 1 ml of said selected amount of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 10μ.

In some embodiments of the coagulation mold, the particle-size distribution D70 of the calcium gluconate powder is between 50μ-200μ.

In some embodiments of the coagulation mold, the particle-size distribution D90 of the calcium gluconate powder is between 500μ-200μ.

In some embodiments of the coagulation mold, the particle-size distribution D90, D80, D70, D60, or D50 of the calcium gluconate powder is about 125μ.

In some embodiments of the coagulation mold, the particle-size distribution of either D50, D60, D70, D80, D90 or D95 of the calcium gluconate powder is between either 50μ-200μ, 60μ-190μ, 70μ-180μ, 80μ-170μ, 90μ-160μ, 100μ-150μ, 110μ-140μ or 120μ-130μ.

In some embodiments of the coagulation mold, the particle-size distribution D99 of the kaolin powder is less than 50μ.

In some embodiments of the coagulation mold, the particle-size distribution D90 of the kaolin powder is less than 20μ or less than 10μ.

In some embodiments of the coagulation mold, the particle-size distribution D50 of the kaolin powder is less than 3μ or between 1μ-3μ.

In some embodiments of the coagulation mold, the particle-size distribution of either D50, D60, D70, D80, D90 or D95 of the calcium gluconate powder is less than either 30μ, 25μ, 20μ,15μ, 10μ, 5μ, 3μ, 2μ, 1μ or less than 0.1μ.

In some embodiments of the coagulation mold, the whole blood being intended to be received therein comprises between 0.04 ml – 0.095 ml of anti-coagulant-containing liquid for every 1 ml of whole blood or in some embodiments about 0.07 ml of anti-coagulant-containing liquid for every 1 ml of whole blood.

In some embodiments, the coagulation mold comprising between 0.6 mg – 3.1 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the coagulation mold comprising about 1.75 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the coagulation mold comprising between 0.8 mg – 2.9 mg, 1 mg – 2.7 mg, 1.2 mg – 2.5 mg, 1.4 mg – 2.3 mg, 1.6 mg – 2.1 mg or 1.8 mg – 2 mg of kaolin powder with every 1 ml of whole blood.

In some embodiments, the mold comprising between 2.5 mg – 8.1 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the mold comprising about 5.3 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the coagulation mold comprising 2.9 mg – 7.7 mg, 3.3 mg – 7.3 mg, 3.7 mg – 6.9 mg, 4.1 mg – 6.5 mg, 4.5 mg – 6.1 mg or 4.9 mg – 5.7 mg of calcium gluconate powder with every 1 ml of whole blood.

In some embodiments of coagulation mold, the volume of the coagulation space is sufficient to receive between 13 ml – 19 ml of whole blood.

In some embodiments of coagulation mold, the volume of the coagulation space is sufficient to receive about 16 ml of whole blood.

Yet another aspect of the present disclosure provides a kit for preparing a coagulated blood mass comprising: calcium gluconate powder; kaolin powder; and a coagulation mold for mixing a selected amount of blood with the calcium gluconate powder and kaolin powder. The calcium gluconate powder and the kaolin powder are characterized by at least one of the following: (i) the particle-size distribution D50 of the calcium gluconate powder is between 50μ-200μ, (ii) wherein said kaolin powder is an amount of between 0.6 mg – 3.1 mg for every 1 ml of said selected amount of whole blood, (iii) wherein said calcium gluconate is an amount of between 2.5 mg – 8.1 mg for every 1 ml of said selected amount of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 10μ.

In some embodiments, the kit comprising tools for withdrawing whole blood and containing it.

In some embodiments, the kit comprising 0.04 ml – 0.095 ml of anti-coagulant-containing liquid for every 1 ml of said selected amount of whole blood. The anti-

coagulant-containing liquid can be contained separately in the kit or within the tool for containing the withdrawn whole blood.

In some embodiments of the kit, said selected amount is between 13 ml – 19 ml.

In some embodiments of the kit, the particle-size distribution D70 of the calcium gluconate powder is between 50µ-200µ.

In some embodiments of the kit, the particle-size distribution D90 of the calcium gluconate powder is between 50µ-200µ.

In some embodiments of the kit, the particle-size distribution D90, D80, D70, D60, or D50 of the calcium gluconate powder is about 125µ.

In some embodiments of the kit, the particle-size distribution of either D50, D60, D70, D80, D90 or D95 of the calcium gluconate powder is between either 50µ-200µ, 60µ-190µ, 70µ-180µ, 80µ-170µ, 90µ-160µ, 100µ-150µ, 110µ-140µ or 120µ-130µ.

In some embodiments of the kit, the particle-size distribution D99 of the kaolin powder is less than 50µ.

In some embodiments of the kit, the particle-size distribution D80 of the kaolin powder is less than 10µ.

In some embodiments of the kit, the particle-size distribution D50 of the kaolin powder is less than 3µ or between 1µ-3µ.

In some embodiments of the kit, the particle-size distribution of either D50, D60, D70, D80, D90 or D95 of the calcium gluconate powder is less than either 30µ, 25µ, 20µ,15µ, 10µ, 5µ, 3µ, 2µ, 1µ or less than 0.1µ.

In some embodiments, the kit comprising between 0.6 mg – 3.1 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the kit comprising about 1.75 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the kit comprising between 0.8 mg – 2.9 mg, 1 mg – 2.7 mg, 1.2 mg – 2.5 mg, 1.4 mg – 2.3 mg, 1.6 mg – 2.1 mg or 1.8 mg – 2 mg of kaolin powder with every 1 ml of whole blood.

In some embodiments, the kit comprising between 2.5 mg – 8.1 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the kit comprising about 5.3 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

In some embodiments, the kit comprising 2.9 mg – 7.7 mg, 3.3 mg – 7.3 mg, 3.7 mg – 6.9 mg, 4.1 mg – 6.5 mg, 4.5 mg – 6.1 mg or 4.9 mg – 5.7 mg of calcium gluconate powder with every 1 ml of whole blood.

In some embodiments, the kit comprising the coagulation mold of any one of the above-described embodiments or any combination thereof. The kaolin powder and calcium gluconate powder are comprised within said coagulation mold.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to better understand the subject matter that is disclosed herein and to exemplify how it may be carried out in practice, embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Figs. 1A-1B are images of the results of the kaolin powder particle size test. **Fig. 1A** shows the result in time zero and **Fig. 1B** shows the result in time 15 minutes.

DETAILED DESCRIPTION

The following are examples of tests with varying parameters of the powders used in the method for forming a coagulated blood mass. These tests are provided to exemplify embodiments and realization of the invention of the present disclosure.

Calcium gluconate (CG) powder test

Success criteria:

Optimal clot is defined by:

- Coagulation time – good coagulation time is under 6 minutes
- Visual clot structure strength – good structure strength is defined as the clot does not break down when extracted and handled by user
- CG residuals – visual assessment of amount of white powder residuals accumulated on the bottom of the clot – 0=no residuals, 5= 100% coverage of surface with residuals

Method:

A standard amount of ACD-A anticoagulant and Kaoiln powder per 1 ml of whole blood was used

- 2 ml of ACD-A for 15ml blood = 0.133ml ACD-A/1ml blood
- 28mg Kaolin powder for 15ml blood = 1.87mg Kaolin/1ml blood

Different amounts of CG where tested – CG particle size after sifting is 125 micron

- 20mg for 15 ml of blood = 1.33mgCG/1ml blood
- 50mg for 15 ml of blood = 3.33mgCG/1ml blood
- 85mg for 15 ml of blood = 5.66mgCG/1ml blood
- 110mg for 15 ml of blood = 7.33mgCG/1ml blood
- 140mg for 15 ml of blood = 9.33mgCG/1ml blood

Coagulation time and clot structure strength were documented. Blood samples of 5 different volunteers were used.

Results:

The following table describes the results of the test:

# volunteer / CG	Coagulation time	Clot strength	Powder residuals
#1/20mg	15	Low	0
#1/50mg	6.8	Medium	0
#1/85mg	4.7	Strong	0
#1/ 110mg	5	Strong	2
#1/ 140mg	4.9	Strong	5
#2/20mg	17	Low	0
#2/50mg	6.7	Medium	0
#2/85mg	4.8	Strong	0
#2/ 110mg	4.7	Strong	2
#2/ 140mg	4.8	Strong	5
#3/20mg	14	Low	0
#3/50mg	5.4	Medium	0
#3/85mg	4.6	Strong	1
#3/ 110mg	5.2	Strong	2
#3/ 140mg	4.7	Strong	5
#4/20mg	12	Low	0

#4/50mg	6	Medium	0
#4/85mg	4.8	Strong	0
#4/ 110mg	4.6	Strong	2
#4/ 140mg	5	Strong	5
#5/20mg	14.5	Low	0
#5/50mg	6.6	Medium	0
#5/85mg	4.6	Strong	0
#5/ 110mg	4.9	Strong	2
#5/ 140mg	5.2	Strong	5
Average/20mg	**14.5**	**Low**	**0**
Average /50mg	**6.3**	**Medium**	**0**
Average /85mg	**4.88**	**Strong**	**0.2**
Average / 110mg	**4.7**	**Strong**	**2**
Average / 140mg	**4.92**	**Strong**	**5**

Table 1

Kaolin powder test

Success criteria:

Optimal clot is defined by:

1. Coagulation time – good coagulation time is under 6 minutes
2. Visual clot structure strength – good structure strength is defined as the clot does not break down when extracted and handled by user

Method:

A standard amount of ACD-A anticoagulant and Calcium Gluconate powder per 1 ml of whole blood was used

- 2 ml of ACD-A for 15ml blood = 0.133ml ACD-A/1ml blood
- 85mg CG powder for 15ml blood = 5.66mg CG/1ml blood

Different amounts of Kaolin where tested – Kaolin particle size is 1-3 micron

- 5mg for 15 ml of blood = 1.33mgCG/1ml blood
- 10mg for 15 ml of blood = 3.33mgCG/1ml blood

- 20mg for 15 ml of blood = 5.66mgCG/1ml blood
- 30mg for 15 ml of blood = 7.33mgCG/1ml blood
- 40mg for 15 ml of blood = 9.33mgCG/1ml blood

Coagulation time and clot structure strength were documented. Blood samples of 5 different volunteers were used.

Results:

The following table describes the results of the test:

# volunteer /Kaolin	Coagulation time	Clot strength
#1/5mg	12	Low
#1/10mg	8.2	Medium
#1/20mg	6.2	Strong
#1/ 30mg	4.7	Strong
#1/ 40mg	4.9	Strong
#2/5mg	14	Low
#2/10mg	8.5	Medium
#2/20mg	5.8	Strong
#2/ 30mg	5.1	Strong
#2/ 40mg	5.5	Strong
#3/5mg	11.5	Low
#3/10mg	7.7	Medium
#3/20mg	5.4	Strong
#3/ 30mg	5.2	Strong
#3/ 40mg	4.8	Strong
#4/5mg	13.4	Low
#4/10mg	8.5	Medium
#4/20mg	6.3	Strong
#4/ 30mg	4.9	Strong
#4/ 40mg	5.2	Strong
#5/5mg	14	Low
#5/10mg	7.3	Medium
#5/20mg	5.4	Strong

#5/ 30mg	5.1	Strong
#5/ 40mg	5.1	Strong
Average/5mg	**12.98**	Low
Average /10mg	**8.04**	Medium
Average /20mg	**5.82**	Strong
Average / 30mg	**5**	Strong
Average / 40mg	**5.1**	Strong

Table 2

Kaolin powder particle size test

Goal

The goal of the test is to establish effective threshold of Kaolin powder to float in water for sufficient time to allow coagulation of blood simultaneously in all volume at the same time.

Background

Blood coagulates rapidly via the extrinsic pathway when Kaolin or other coagulation initiator (such as glass) comes in contact with factor XI. The more Kaolin particles that come in contact with blood for more time, the higher probability of contact. Kaolin is a clay mineral that typically sink in water due to its weight. If Kaolin particles will be able to freely float in liquid it will have higher effect of coagulation, achieving an evenly distributed coagulation that starts at once in all the blood volume, similar in the upper part of the blood volume as in the lower part of the blood volume etc. The idea is that if kaolin particles are sufficiently small, they can float in the blood for a sufficient duration before they start to sink to allow homogenic coagulation initiation and acceleration, achieving a homogenic clot.

Success criteria

- Kaolin particles float in liquid for 15 minutes without sinking

Method

2 sizes of particles were tested

- Smaller than 25 micron
- Larger than 25 micron

For each group of particles size, 50mg of powder was placed inside a clear glass vessel with 160ml of purified water. Both glass vessels were mixed for 5 seconds with a steering spoon. At time points 0 and 15 minutes the vessels were visually assessed for the following parameters

1. Water color and transparency (from 10: white/non transparent to 1: Clear/Fully Transparent)
2. Accumulation of kaolin powder on the bottom of the vessel (From 10: No accumulation to 1: fully accumulated)

Results

The following table describes the results of the test (and also can be seen in **Figs. 1A-1B**):

#	Water color&transparency	Accumulation on the bottom
Time0 Cup <25micron	10	10
Time0 Cup> 25micron	10	10
Time15min Cup<25micron	10	2
Time15min Cup<25micron	10	4

Table 3

Conclusions

Kaolin particle size lower that 25micron float in water and thus in blood for 15 minutes without sinking while kaolin particle larger than 25 micron will sink within timeframe of 15 minutes.

CLAIMS:

1. A method for preparing a coagulated blood mass, for use in a treatment of a subject, comprising:

mixing an amount of whole blood with calcium gluconate powder and kaolin powder; and

allowing the whole blood to coagulate to form the coagulated blood mass;

the method is further characterized by at least one of: (i) the particle-size distribution D50 of the calcium gluconate powder is between 50µ-200µ, (ii) wherein said mixing comprises mixing between 0.6 mg – 3.1 mg of kaolin powder with every 1 ml of whole blood, (iii) wherein said mixing comprises mixing between 2.5 mg – 8.1 mg of calcium gluconate powder with every 1 ml of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 25µ.

2. The method of claim 1, wherein the particle-size distribution D70 of the calcium gluconate powder is between 50µ-200µ.

3. The method of claim 1 or 2, wherein the particle-size distribution D90 of the calcium gluconate powder is between 50µ-200µ.

4. The method of any one of claims 1-3, wherein the particle-size distribution D50 of the calcium gluconate powder is about 125µ.

5. The method of any one of claims 1-4, comprising withdrawing said whole blood from the subject.

6. The method of any one of claims 1-5, comprising, prior to said mixing, introducing anti-coagulant-containing liquid to said amount of whole blood.

7. The method of claim 6, wherein said introducing comprises introducing between 0.04 ml – 0.095 ml of anti-coagulant-containing liquid for every 1 ml of whole blood.

8. The method of claim 7, wherein said introducing comprises introducing about 0.07 ml of anti-coagulant-containing liquid for every 1 ml of whole blood.

9. The method of any one of claims 1-8, wherein said mixing is performed within a coagulation mold.

10. The method of any one of claims 1-9, wherein said mixing comprises mixing between 0.6 mg – 3.1 mg of kaolin powder with every 1 ml of whole blood.

11. The method of claim 10, wherein said mixing comprises mixing about 1.75 mg of kaolin powder with every 1 ml of whole blood.

12. The method of any one of claims 1-11, wherein said mixing comprises mixing between 2.5 mg – 8.1 mg of calcium gluconate powder with every 1 ml of whole blood.

13. The method of claim 12, wherein said mixing comprises mixing about 5.3 mg of calcium gluconate powder with every 1 ml of whole blood.

14. The method of any one of claims 1-13, wherein the particle-size distribution D99 of the kaolin powder is less than 50μ.

15. The method of any one of claims 1-14, wherein the particle-size distribution D80 of the kaolin powder is less than 25μ.

16. The method of any one of claims 1-15, wherein the particle-size distribution D45 of the kaolin powder is less than 3μ.

17. A coagulation mold comprising:

a coagulation space defined by a base surrounded by upwardly rising walls and configured to receive a selected amount of whole blood;

said coagulation space comprises calcium gluconate powder and kaolin powder;

wherein said coagulation mold is characterized by at least one of: (i) the particle-size distribution D50 of the calcium gluconate powder is between 50μ-200μ, (ii) wherein said kaolin powder is an amount of between 0.6 mg – 3.1 mg for every 1 ml of said selected amount of whole blood, (iii) wherein said calcium gluconate is an amount of between 2.5 mg – 8.1 mg for every 1 ml of said selected amount of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 10μ.

18. The coagulation mold of claim 17, wherein the particle-size distribution D70 of the calcium gluconate powder is between 50μ-200μ.

19. The coagulation mold of claim 17 or 18, wherein the particle-size distribution D90 of the calcium gluconate powder is between 50μ-200μ.

20. The coagulation mold of any one of claims 17-19, wherein the particle-size distribution D50 of the calcium gluconate powder is about 125μ.

21. The coagulation mold of any one of claims 17-20, wherein the particle-size distribution D99 of the kaolin powder is less than 50μ.

22. The coagulation mold of any one of claims 17-21, wherein the particle-size distribution D80 of the kaolin powder is less than 25μ.

23. The coagulation mold of any one of claims 17-22, wherein the particle-size distribution D45 of the kaolin powder is less than 3μ.

24. The coagulation mold of any one of claims 17-23, wherein the whole blood being intended to be received therein comprises between 0.04 ml – 0.095 ml of anti-coagulant-containing liquid for every 1 ml of whole blood.

25. The coagulation mold of any one of claims 17-24, comprising between 0.6 mg – 3.1 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

26. The coagulation mold of claim 25, comprising about 1.75 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

27. The coagulation mold of any one of claims 17-26, comprising between 2.5 mg – 8.1 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

28. The coagulation mold of claim 27, comprising about 5.3 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

29. The coagulation mold of any one of claims 17-28, wherein the volume of the coagulation space is sufficient to receive between 13 ml – 19 ml of whole blood.

30. A kit for preparing a coagulated blood mass comprising:

calcium gluconate powder;

kaolin powder;

a coagulation mold for mixing a selected amount of blood with the calcium gluconate powder and kaolin powder;

the calcium gluconate powder and the kaolin powder are characterized by at least one of the following: (i) the particle-size distribution D50 of the calcium gluconate powder is between 50μ-200μ, (ii) wherein said kaolin powder is an amount of between 0.6 mg – 3.1 mg for every 1 ml of said selected amount of whole blood, (iii) wherein said calcium gluconate is an amount of between 2.5 mg – 8.1 mg for every 1 ml of said selected amount of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 10μ.

31. The kit of claim 30, comprising tools for withdrawing whole blood and containing it.

32. The kit of claim 30 or 31, comprising 0.04 ml – 0.095 ml of anti-coagulant-containing liquid for every 1 ml of said selected amount of whole blood.

33. The kit of any one of claims 30-32, wherein said selected amount is between 13 ml – 19 ml.

34. The kit of any one of claims 30-33, wherein the particle-size distribution D70 of the calcium gluconate powder is between 50µ-200µ.

35. The kit of any one of claims 30-34, wherein the particle-size distribution D90 of the calcium gluconate powder is between 50µ-200µ.

36. The kit of claim 35, wherein the particle-size distribution D50 of the calcium gluconate powder is about 125µ.

37. The kit of any one of claims 30-36, wherein the particle-size distribution D99 of the kaolin powder is less than 50µ.

38. The kit of any one of claims 30-37, wherein the particle-size distribution D80 of the kaolin powder is less than 25µ.

39. The kit of any one of claims 30-38, wherein the particle-size distribution D45 of the kaolin powder is less than 3µ.

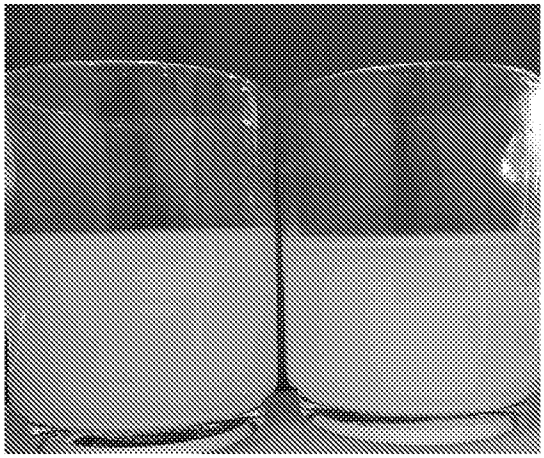
40. The kit of any one of claims 30-39, comprising between 0.6 mg – 3.1 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

41. The kit of claim 40, comprising about 1.75 mg of kaolin powder for every 1 ml of said selected amount of whole blood.

42. The kit of any one of claims 30-41, comprising between 2.5 mg – 8.1 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

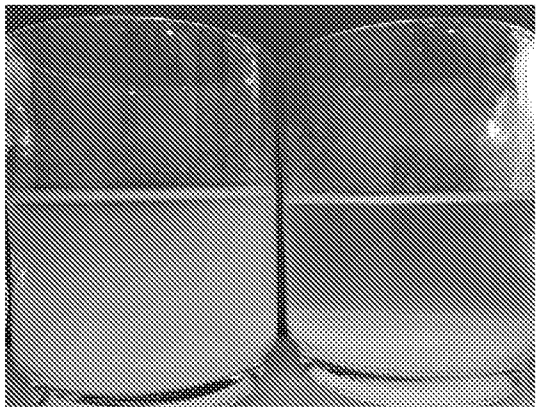
43. The kit of claim 42, comprising about 5.3 mg of calcium gluconate powder for every 1 ml of said selected amount of whole blood.

44. The kit of any one of claims 30-43, comprising the coagulation mold of any one of claims 17-29, said kaolin powder and calcium gluconate powder are comprised within said coagulation mold.



Time zero – Left<25micron right>25micron

Fig. 1A



Time 15min - Left<25micron right>25micron

Fig. 1B

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2023/050083

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61J1/00 A61K31/00 A61K35/14 A61M1/00 A61P7/00
ADD.

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 A61J A61K A61P

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 practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/086848 A2 (KUSHNIR ALON [IL]; KUSHNIR IGAL [IL]) 5 August 2010 (2010-08-05) pages 32-35 and 40, table 3 -/--	1-44

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" 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

"X" 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

"Y" 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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2023	17/03/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Basso, Veronica

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2023/050083

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kushnir Igal ET AL: "Efficacy and Safety of a Novel Autologous Wound Matrix in the Management of Complicated, Chronic Wounds: A Pilot Study", Index Wounds, 1 September 2016 (2016-09-01), XP055912334, Retrieved from the Internet: URL:https://www.hmpgloballearningnetwork.com/site/wounds/article/efficacy-and-safety-novel-autologous-wound-matrix-management-complicated-chronic-wounds [retrieved on 2022-04-13] Abstract, page 3	1-44
X	SERENA THOMAS E ET AL: "The safety of an autologous whole blood clot product applied to full thickness dermal wounds in a porcine model for up to 18 days", CHRONIC WOUND CARE MANAGEMENT AND RESEARCH, vol. Volume 6, 19 June 2019 (2019-06-19), pages 39-49, XP093029883, DOI: 10.2147/CWCMR.S189836 Retrieved from the Internet: URL:https://www.dovepress.com/getfile.php?fileID=50610> page 40, page 41, left column	1-44

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Information on patent family members

International application No
PCT/IL2023/050083

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