



(51) International Patent Classification:

A61J 1/00 (2006.01)

A61M 1/00 (2006.01)

A61K 31/00 (2006.01)

A61P 7/00 (2006.01)

A61K 35/14 (2006.01)

SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(21) International Application Number:

PCT/IL2023/050083

(22) International Filing Date:

25 January 2023 (25.01.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

290122

25 January 2022 (25.01.2022)

IL

(71) Applicant: REDDRESS LTD. [IL/IL]; 11 Shkedim Street, 3701142 Pardes Hana (IL).

(72) Inventor: KUSHNIR, Alon; 6 Haarava Street, 3780800 Givat Ada (IL).

(74) Agent: COHN, Lior; c/o COHN, DE VRIES, STADLER & CO., P.O.B 10278, 6110103 Tel Aviv (IL).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI,

(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 The coagulated blood mass is prepared from whole blood being withdrawn from a subject.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 these 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.



WO 2023/144818 A1

- 1 -

## 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

5           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

10

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.

### 15   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  
20 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  
25 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

- 2 -

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  
5 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  
10 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  
15 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 50 $\mu$ -200 $\mu$ , (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-  
20 size distribution D50 of the kaolin powder is less than 20 $\mu$ .

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 125 $\mu$  then 50% of the particles are having a volumetric size equal or less to 125 $\mu$ . By maintaining only particles with size that is relatively small,  
25 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 homogenous.

30 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.

- 3 -

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

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 2 $\mu$ .

5 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 120-125 $\mu$  particles. Particle size of less than 120-125 $\mu$  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  
10 coagulated blood mass.

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

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

15 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 125 $\mu$ , then the value should be considered as 100 $\mu$ -150 $\mu$ .

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 50 $\mu$ -  
20 200 $\mu$ , 60 $\mu$ -190 $\mu$ , 70 $\mu$ -180 $\mu$ , 80 $\mu$ -170 $\mu$ , 90 $\mu$ -160 $\mu$ , 100 $\mu$ -150 $\mu$ , 110 $\mu$ -140 $\mu$  or 120 $\mu$ -130 $\mu$ .

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

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

25 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.

30 In some embodiments of the method, the particle-size distribution D80 of the kaolin powder is less than 25 $\mu$ .

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

- 4 -

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 30 $\mu$ , 25 $\mu$ , 20 $\mu$ , 15 $\mu$ , 10 $\mu$ , 5 $\mu$ , 3 $\mu$ , 2 $\mu$ , 1 $\mu$  or less than 0.1 $\mu$ .

In some embodiments, the method comprising withdrawing said whole blood  
5 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  
10 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  
15 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  
20 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  
25 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.

30 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.

- 5 -

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  
5 comprises at least one of the following: (i) the particle-size distribution D50 of the calcium gluconate powder between  $50\mu$ - $200\mu$ , (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  
10 powder is less than  $10\mu$ .

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

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

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

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  $50\mu$ - $200\mu$ ,  $60\mu$ - $190\mu$ ,  $70\mu$ - $180\mu$ ,  $80\mu$ - $170\mu$ ,  $90\mu$ - $160\mu$ ,  $100\mu$ - $150\mu$ ,  $110\mu$ - $140\mu$  or  $120\mu$ -  
20  $130\mu$ .

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

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

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

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  $30\mu$ ,  $25\mu$ ,  $20\mu$ ,  $15\mu$ ,  $10\mu$ ,  $5\mu$ ,  $3\mu$ ,  $2\mu$ ,  $1\mu$  or less than  $0.1\mu$ .

30 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.

- 6 -

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.

5 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.

10 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.

15 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  
20 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 50 $\mu$ –200 $\mu$ , (ii) wherein said kaolin powder is an  
25 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 10 $\mu$ .

30 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-

- 7 -

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  
5 gluconate powder is between 50 $\mu$ -200 $\mu$ .

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

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

10 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 50 $\mu$ -200 $\mu$ , 60 $\mu$ -190 $\mu$ , 70 $\mu$ -180 $\mu$ , 80 $\mu$ -170 $\mu$ , 90 $\mu$ -160 $\mu$ , 100 $\mu$ -150 $\mu$ , 110 $\mu$ -140 $\mu$  or 120 $\mu$ -130 $\mu$ .

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

15 In some embodiments of the kit, the particle-size distribution D80 of the kaolin powder is less than 10 $\mu$ .

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

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

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  
25 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  
30 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.

- 8 -

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  
5 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  
10 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.

15

### 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.

20

#### **Calcium gluconate (CG) powder test**

##### Success criteria:

Optimal clot is defined by:

- Coagulation time – good coagulation time is under 6 minutes
- 25 • 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

30 Method:

- 9 -

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
- 5 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
- 10 • 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                |

- 10 -

|                                  |             |               |            |
|----------------------------------|-------------|---------------|------------|
| #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          |
| <b>Average/20mg</b>              | <b>14.5</b> | <b>Low</b>    | <b>0</b>   |
| <b>Average /50mg</b>             | <b>6.3</b>  | <b>Medium</b> | <b>0</b>   |
| <b>Average /85mg</b>             | <b>4.88</b> | <b>Strong</b> | <b>0.2</b> |
| <b>Average /</b><br><b>110mg</b> | <b>4.7</b>  | <b>Strong</b> | <b>2</b>   |
| <b>Average /</b><br><b>140mg</b> | <b>4.92</b> | <b>Strong</b> | <b>5</b>   |

Table 1

**Kaolin powder test****Success criteria:**

- 5 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:**

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

- 11 -

- 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  
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        |

- 12 -

|                       |              |        |
|-----------------------|--------------|--------|
| #5/ 30mg              | 5.1          | Strong |
| #5/ 40mg              | 5.1          | Strong |
| <b>Average/5mg</b>    | <b>12.98</b> | Low    |
| <b>Average /10mg</b>  | <b>8.04</b>  | Medium |
| <b>Average /20mg</b>  | <b>5.82</b>  | Strong |
| <b>Average / 30mg</b> | <b>5</b>     | Strong |
| <b>Average / 40mg</b> | <b>5.1</b>   | Strong |

Table 2

**Kaolin powder particle size test****5 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**

- 10 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
- 15 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.

**20 Success criteria**

- Kaolin particles float in liquid for 15 minutes without sinking

**Method**

2 sizes of particles were tested

- Smaller than 25 micron
- 25 • Larger than 25 micron

- 13 -

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

- 5      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

- 10      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<br>Cup<br><25micron | 10                       | 10                         |
| Time0<br>Cup><br>25micron | 10                       | 10                         |
| Time15min<br>Cup<25micron | 10                       | 2                          |
| Time15min<br>Cup>25micron | 10                       | 4                          |

Table 3

#### 15 Conclusions

Kaolin particle size lower than 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.

- 14 -

**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
  - 5 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 50 $\mu$ -200 $\mu$ , (ii) wherein said mixing comprises mixing between 0.6 mg – 3.1 mg of kaolin powder with every 1 ml of
  - 10 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 25 $\mu$ .
2. The method of claim 1, wherein the particle-size distribution D70 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ .
- 15 3. The method of claim 1 or 2, wherein the particle-size distribution D90 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ .
4. The method of any one of claims 1-3, wherein the particle-size distribution D50 of the calcium gluconate powder is about 125 $\mu$ .
5. The method of any one of claims 1-4, comprising withdrawing said whole blood
- 20 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.
- 25 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
- 30 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.

- 15 -

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.
- 5 14. The method of any one of claims 1-13, wherein the particle-size distribution D99 of the kaolin powder is less than 50 $\mu$ .
15. The method of any one of claims 1-14, wherein the particle-size distribution D80 of the kaolin powder is less than 25 $\mu$ .
16. The method of any one of claims 1-15, wherein the particle-size distribution D45  
10 of the kaolin powder is less than 3 $\mu$ .
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;  
15 wherein said coagulation mold is characterized by at least one of: (i) the particle-size distribution D50 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ , (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)  
20 the particle-size distribution D50 of the kaolin powder is less than 10 $\mu$ .
18. The coagulation mold of claim 17, wherein the particle-size distribution D70 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ .
19. The coagulation mold of claim 17 or 18, wherein the particle-size distribution D90 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ .
- 25 20. The coagulation mold of any one of claims 17-19, wherein the particle-size distribution D50 of the calcium gluconate powder is about 125 $\mu$ .
21. The coagulation mold of any one of claims 17-20, wherein the particle-size distribution D99 of the kaolin powder is less than 50 $\mu$ .
22. The coagulation mold of any one of claims 17-21, wherein the particle-size  
30 distribution D80 of the kaolin powder is less than 25 $\mu$ .
23. The coagulation mold of any one of claims 17-22, wherein the particle-size distribution D45 of the kaolin powder is less than 3 $\mu$ .

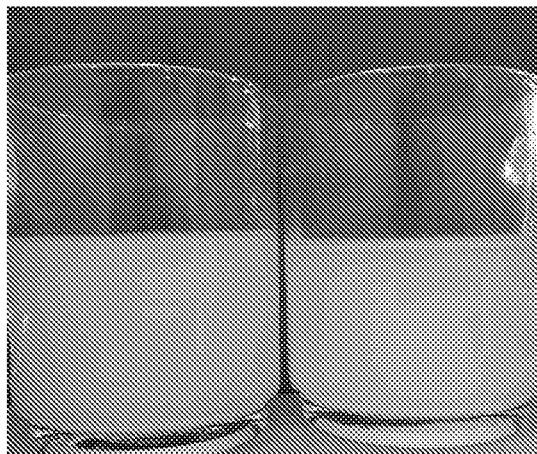
- 16 -

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 –  
5 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  
10 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.
- 15 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;
- 20 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 50 $\mu$ -200 $\mu$ , (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  
25 amount of whole blood, or (iv) the particle-size distribution D50 of the kaolin powder is less than 10 $\mu$ .
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.  
30
33. The kit of any one of claims 30-32, wherein said selected amount is between 13 ml – 19 ml.

- 17 -

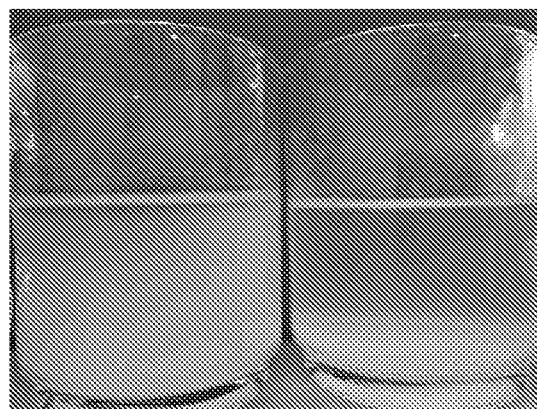
34. The kit of any one of claims 30-33, wherein the particle-size distribution D70 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ .
35. The kit of any one of claims 30-34, wherein the particle-size distribution D90 of the calcium gluconate powder is between 50 $\mu$ -200 $\mu$ .
- 5 36. The kit of claim 35, wherein the particle-size distribution D50 of the calcium gluconate powder is about 125 $\mu$ .
37. The kit of any one of claims 30-36, wherein the particle-size distribution D99 of the kaolin powder is less than 50 $\mu$ .
38. The kit of any one of claims 30-37, wherein the particle-size distribution D80 of  
10 the kaolin powder is less than 25 $\mu$ .
39. The kit of any one of claims 30-38, wherein the particle-size distribution D45 of the kaolin powder is less than 3 $\mu$ .
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.
- 15 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  
20 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.

1/1



Time zero – Left<25micron right>25micron

**Fig. 1A**



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

**Fig. 1B**