METHOD OF MAKING FIBRINOGEN PARTICLES SUITED FOR INTRAVENOUS INJECTIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method for producing synthetic protein particles, which are particularly used to significantly reduce a patient's blood loss during surgical and medical treatments, and which are used to help make a quick recovery from such surgical and medical treatments. More particularly, the present invention relates to a method for making fibrinogen particles suited for intravenous injections.

2. Description of the Prior Art

It is known that many surgical and medical procedures such as a cancer operation may cause a mass amount of blood loss, which sometimes may put patients in danger of death, especially to those who lack of adequate platelets in their blood. One approach to remedy the blood loss is using a blood transfusion. However, this may introduce fatal diseases such as HIV virus or hepatitis virus. Accordingly, in the field of clinical medicine, there is a strong need to provide a substance which is helpful in aggregation of blood or substantially functions as a platelet in human blood. Such substance must meet the following requirements. First, the particle size of such substance must as small as possible such that it can flow into the capillary vessel network of a human body. The maximum diameter of such substance cannot exceed 7 micrometers, which is, on average, the diameter of human capillary vessel. Secondly, such substance should not aggregate itself in human blood or

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initiate blood aggregation of blood cells, but help the blood aggregation at the site of a wound. More specifically, such substance must encompass the ability to participate or promote blood clotting at the site of a wound.

K.J. Widder et al. in "Magnetically Responsive Microspheres and Other Carriers for the Biophysical Targeting of Antitumor Agents", Advances in Pharmacology and Chemotherapy, vol.6, pp. 213-271, disclosed microspheres made from proteins, which are synthesized by using emulsification of an aqueous protein solution in oil, followed by heat treatment or extensive polymerization. However, the lifetime of the microspheres made according Widder's method is short since the protein is subject to undesirable denaturing reactions during the making of such microspheres. In practice, the synthesized platelet-like particles must be preserved at least for one day in human blood vessels.

Oppenheim et al., in U.S.Pat.No.4,107,288 entitled "Injectable compositions, nanoparticles useful therein, and process of manufacturing same", disclosed a method of manufacturing particles comprising a crosslinked matrix of macromolecules of natural origin. In this method, the protein solutions were pushed to the limit of desolubilization by adjusting the concentration of a desolubilization agent with a contrasting resolubilization agent. This process starts with an unstable conditionand proceeds with extensive polymerization which causes the spheres to form, with no control over the extent of aggregation. Aggregates greater than 7 microns in diameter can clog blood vessels and are therefore dangerous for intravascular applications. There is therefore a need for particles that do not aggregate and are considerably smaller than 7 microns in diameter.

SUMMARY OF THE INVENTION

The main objective of the claimed invention is to provide a method for making fibrinogen particles, which are suited for intravenous injection applications.

In accordance with the present invention, the microspheres formed in the presence of cross-linking agents have an average diameter of less than 1 micron.

In accordance with the present invention, the fibrinogen may be used as a targeting molecule, which can bring protein particles to the site of a wound, thereby reducing blood loss.

In accordance with the present invention, the synthesized substance functions as a platelet in human blood. The synthesized substance of this invention is also referred to as fibrinoplate or platelet aggregation substitute.

In accordance with the present invention, the composition of the substance includes (parts in weight):

Human albumin: 90-100 parts

Fibrinogen: 0.02-6 parts

The substance of this invention is made according to the following procedures:

1. preparing protein particle suspension solution, wherein:
   a. dissolving protein powders in a buffer solution having suitable osmolarity;
   b. adding surfactant into prepared solution of step a;
   c. adding cross-linking agents into mixture solution of step c; and
   d. adding alcohol solution into mixture solution of step

c.

A nebulous protein particle suspension solution, which is suited for intravenous injection applications and will not clog the blood vessel, is thus prepared.

In accordance with the present invention, the protein solution is human albumin solution.

In accordance with the present invention, the surfactant is a cleaning agent or a detergent.

In accordance with the present invention, the surfactant may be selected from the group consisting of sodium tetradecyl sulfate, Tween-80, and TritonX-151.

In accordance with the present invention, the cross-linking agent is a covalent small molecule such as glutaraldehyde.

In accordance with the present invention, the pH of the buffer solution is between 4 and 8.

In accordance with the present invention, the osmolarity is 14-680 mOsm.

2. After preparing the above nebulous protein particle suspension solution, surface protein is then added into the suspension solution. By doing this, the un-reacted portions on the micro-spheres will transform the surface protein into fibrinogen through covalence bonding.

3. The human albumin sphere suspension solution containing surface protein is then treated in the form of powders via dilution, filtration, dialysis, diafiltration,

centrifugation, electrophoresis, column chromatography, vacuum freeze-dry, and powder reconstitution. In some cases, addition or change of buffer solution or addition of preservative is needed, which is known in the art.

Accordingly, the present invention is concerned with the making of fibrinogen particles, which are suited for intravenous injections, wherein the composition of protein particle suspension solution will not clog the blood vessel, but aggregate at the site of a wound, and the resulting protein particle is made from human albumin cross-linking fibrinogen having an average diameter of less than 7 micrometers.

The present invention is concerned with frozen protein particle composition, which is reconstituted through bio-soluble liquids and is suited for intravenous injections. The protein composition of this invention does not clog blood vessels (except at the site of a wound). The resulting protein particle is made from human albumin cross-linking fibrinogen having an average diameter of less than 7 micrometers.

The present invention is concerned with protein particle suspension solution, which is suited for intravenous injections. The protein composition of this invention does not clog blood vessels (except at the site of a wound). The resulting protein composition is made at the surface of spheres from human albumin cross-linking fibrinogen with covalence bonding. The resulting protein particle has an average diameter of less than 7 micrometers.

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vessels (except at the site of a wound). The resulting protein particle composition is made at the surface of spheres from human albumin cross-linking fibrinogen with covalence bonding, wherein the fibrinogen encapsulates the human albumin. The resulting protein particle has an average diameter of less than 7 micrometers.

In accordance with another aspect of the present invention, the present invention provides a new utility of such protein particle to human body through intravenous injections. The protein particles can migrate to a new wound and help aggregation, thereby reducing blood loss.

Further, the protein particles of this invention can be used on trauma patients who are in danger of mass blood loss.

Other objects, advantages and novel features of the invention will become more clearly and readily apparent from the following detailed description when taken in conjunction with the accompanying drawings.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is illustrated by, but by no means limited to, the following examples. Although the following experiments use human serum albumin (HSA) as a starting material, it is understood that a number of common human protein such as immunoglobulin, myoglobin, collagen, or gelatin, can be used as constituents of the spheres.

1. Experiment One: The formation of spheres without fibrinogen. Powder human serum albumin (HSA) was dissolved in normal saline (NS, or 0.9% sodium

chloride solution) to form solutions ranging from 5% to 20% (weight per volume) containing also a surfactant either sodium tetradecyl sulphate or Tween-80, or Triton X-151, in concentrations ranging from 0.05 mg/ml to 15 mg/ml. To a series of tubes containing 2ml of protein solution with various concentrations of surfactant, 2ml of glutaraldahyde, diluted with normal slaine, in concentrations ranging from 0.01% to 3.0% was added to the protein solution. After 10 minutes, about 10 ml ethanol (about 80% diluted in water) was added. Turbidity was observed immediately. After another 10 minutes, the turbid suspensions were diluted with five volumes of saline to see if they redissolved, which they did not. Thereafter, the particles were observed under ×1000 phase microscopy.

It was discovered that the average diameter of the spheres was less than 1 micron.

It was also discovered that Triton X-151 in the range of 1-2.5 mg/ml with HSA concentration in the range of 100-200 mg/ml gave the best preparations. It was also noted that sodium tetradecyl sulphate in the range of 1 to 12 mg/ml produced the most homogeneous preparations in terms of size distribution.

2. Experiment Two: Binding of a Targeting Agent, Fibrinogen, to protein spheres. Radiolabeled human serum albumin (with iodine-125) was used to prepare spheres using the concentrations of Experiment One. It was found that over 95% of the proteins were incorporated into spheres, as measured by the number of radioactive counts in the pellet versus the

supernatant, after centrifugation to sediment the spheres. In creasing the osmolarity of the solutions to 1.8% sodium chloride solution (twice that of normal saline) or decreasing the osmolarity to 0.09% sodium chloride solution had no significant effect on the yield of spheres. Decreasing the pH of the buffer to less than 4 required more alcohol than a pH of 8 to form spheres and yield was slightly diminished in pH of less than 4.

The experiment was repeated suing the optimal condition, but with fluorescein isothiocyanate labeled human serum albumin. Using the optimal condition as listed in Experiment One, fibrinogen (1mg/ml) was added at various times to the sphere suspensions after appearance of turbidity. It was noted that the spheres were stable and the best preparations were obtained by adding fibrinogen within 15 minutes of formation of spheres.

Further treatment of the sphere suspensions may be desirable, such as dilution, filtration, dialysis, diafiltration, centrifugation, electrophoresis, column chromatography, addition or change of buffers or preservative, lyophilization, and the reconstitution of lyophilized powder with an aqueous fluid (such as water or normal saline or lactated Ringer Solution or sugar containing fluid) to result in a physiologically compatible suspension ready for adminstration to a subject in need.

Rabbits were rendered thrombocytopenic according to the method of McGill et al., "Platelet membrane vesicles reduced microvascular bleeding times in thrombocytopenic rabbits, J. Lab Clin Med 1987; 109:127-33. The typical bleeding time in

ten rabbits after 70 mg/kg of Busulfan treatment was 597 seconds. Upon injection of 1 mg/kg of the fibrinogen coated spheres (reconstituted with normal saline from lyophilized powder), the bleeding time was reduced to 115 seconds (standard deviation of 43 seconds). Examination of the section of the ear wound under fluorescent microscopy showed fluorescent particles within the clot. Thrombocytopenic rabbits infused with fluorescent spheres without fibrinogen; or with normal saline, or with a fibrinogen solution (1 mg/ml) did not show improvement in bleeding clot in the ear. This showed that the fibrinogen molecules could act as a homing device to bring particles to an area of active clot formation, resulting in improved bleeding time and less blood loss.

We expect these particles to be equally effective in trauma or surgical patients who may not be thrombocytopenic but can benefit from a faster clotting time. We expect these particles to be particularly useful when large number of patients need to be treated but platelet donors are not hard to find, such as in battle field conditions or after nuclear war events.

Those skilled in the art will readily observe that numerous modification and alterations of the device may be made while retaining the teachings of the invention. Accordingly, the above disclosure should be construed as limited only by the metes and bounds of the appended claims.

CLAIMS

What is claimed is:

1. A method of making protein particles suited for intravenous injections, comprising:

a. dissolving protein powders in a buffer of suitable osmolarity;

b. adding a surfactant selected from a group consisting of sodium tetradecyl sulfate, Tween-80, and TritonX-151 into said protein solution;

c. adding a cross-linker solution including a covalent small molecule such as glutaraldehyde to said mixture of protein and surfactant; and

d. adding an alcohol to said mixture of protein, surfactant, and cross-linker solution.

3. The method as claimed in claim 1 wherein the protein solution is a human serum albumin solution.

4. The method as claimed in claim 1 wherein the surfactant is a detergent.

4. The method as claimed in claim 1 wherein the buffer has a pH of 4-8.

5. The method as claimed in claim 1 wherein the osmolarity of the solutions ranges from 14 to 680 mOsm.

6. The method as claimed in claim 1 wherein the alcohol is ethanol.

7. The method as claimed in claim 1 wherein the particles in the suspension are rendered into powder form.

8. The method as claimed in claim 7 wherein further treatments include filtration, drying, and reconstitution.

9. The method as claimed in claim 1 further comprising adding surface protein to the sphere suspension.

10. The method as claimed in claim 9 wherein the surface protein is human fibrinogen.

11. The method as claimed in claim 9 wherein the protein solution is human serum albumin solution.

12. The method as claimed in claim 9 wherein the surfactant is detergent.

13. The method as claimed in claim 12 wherein the surfactant is selected from a group consisting of sodium tetradecyl sulfate, Tween-80, and TritonX-151.

14. The method as claimed in claim 9 wherein the cross-linker molecule is a bivanently reactive small molecule including glutaraldehyde.

15. The method as claimed in claim 9 wherein the alcohol is ethanol.

16. The method as claimed in claim 9 wherein the pH of the solutions ranges from 4 to 8.

17. The method as claimed in claim 9 wherein the osmolarity of the solutions ranges from 14 to 680 mOsm.

18. The method as claimed in claim 9 wherein the particles in the suspension are rendered into powder form.

19. The method as claimed in claim 9 wherein further treatments include filtration, drying, and reconstitutio.