Summary ~~of Invention~~

In one aspect, the present disclosure refers to a method of forming a keratin filament network, comprising:

(i) dialysing a solubilized keratin solution in a dialysis buffer solution at a pH of about 2.5 to about 5.5 to obtain purified keratin;

(ii) mixing the purified keratin with a salt in acidic buffer solution; and

(iii) drying the solution of step (ii) to form the keratin filament network.

Advantageously, the disclosed method may use solubilized human hair keratins which can be extracted from any keratin source. One example of a keratin source could be human hair waste which is highly abundant and easily collected from hairdressers and salons. Personalized keratins can also be obtained by collecting one's own hair.

Also advantageously, the method allows for high throughput and ease of up-scaling. The yield of keratins extracted from human hair may be as high as 51%. This allows for up-scaling for downstream production more feasible as large quantities of hair keratins can be obtained, compared to recombinant technologies for protein production, which produce limited quantities at high cost.

Further advantageously, the disclosed method is less complicated and less laborious. The disclosed method may achieve well defined, self-assembled keratin fibers and is straightforward. No additional purification steps are required before conducting dialysis and initiating self-assembly of the intermediate filaments proteins.

Also advantageously, the disclosed method may provide self-assembled filamentous mesh-like networks, instead of sporadic random fibers.

~~In another aspect, the present disclosure refers to a keratin filament network comprising at least 2 Type I human hair keratins and at least 2 Type II human hair keratins.~~

~~In yet another aspect, the present disclosure refers to a keratin filament network obtained by the method as disclosed herein.~~

Advantageously, the keratin filament networks may be useful in cosmetic and personal care applications, e.g. hair surface enhancement template and formulated keratin moisturizing cream for managing skin conditions, and research and medical applications, e.g. coatings for

CLAIMS

1. A method of forming a keratin filament network, comprising:
   (i) dialysing a solubilized keratin solution in a dialysis buffer solution at a pH of ~~about~~ 2.5 to ~~about~~ 5.5 to obtain purified keratin;
   (ii) mixing the purified keratin with a salt in acidic buffer solution; and
   (iii) drying the solution of step (ii) to form the keratin filament network.

2. The method of claim 1, wherein the method comprises self-assembly of the keratin filament network.

3. The method of claim 1 or 2, wherein the dialysis buffer solution comprises citric acid, acetic acid, formic acid, ascorbic acid, benzoic acid, propionic acid, sorbic acid, maleic acid, gallic acid and/or lactic acid.

4. The method of anyone of the preceding claims, wherein the dialysis buffer solution further comprises a denaturing agent.

5. The method of claim 4, wherein the denaturing agent is urea or guanidine HCl.

6. The method of claim 4 or 5, wherein the starting concentration of the denaturing agent in the dialysis buffer solution is in the range of ~~about~~ 6M to ~~about~~ 10M.

7. The method of any one of the preceding claims, wherein step (i) is performed for at least 2 hours.

8. The method of any one of the preceding claims, wherein step (i) is repeated more than once.

9. The method of claim 8, wherein the concentration of denaturing agent in each repeated step (i) is lower than the concentration of denaturing agent in the preceding step (i).

10. The method of claim 8 or 9, wherein the concentration of denaturing agent in each repeated step (i) is about half the concentration of denaturing agent in the preceding step (i).

11. The method of anyone of the preceding claims, wherein the dialysis buffer solution further comprises a reducing agent.

12. The method of claim 11, wherein the concentration of reducing agent in the dialysis buffer solution is ~~about~~ 0.5 mM to ~~about~~ 10mM.

13. The method of claim 11 or 12, wherein the reducing agent is selected from the group consisting of dithiothreitol (DTT), beta-mercaptoethanol, tris(2-carboxyethyl)phosphine), and dithioerythritol (DTE).

14. The method of anyone of the preceding claims, wherein the acidic buffer solution comprises citric acid, acetic acid, formic acid, ascorbic acid, benzoic acid, propionic acid, sorbic acid, maleic acid, gallic acid and/or lactic acid.

15. The method of anyone of the preceding claims, wherein the concentration of the salt in the acidic buffer solution is ~~about~~ 1mM to ~~about~~ 40 mM.

16. The method of anyone of the preceding claims, wherein the salt is selected from the group consisting of carbonate salts, sulfate salts, thiosulfate salts, phosphate salts, fluoride salts and chloride salts.

17. The method of anyone of the preceding claims, wherein the salt is selected from the group consisting of lithium chloride, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulfate, ammonium sulfate, potassium carbonate, and calcium sulfate.

18. The method of anyone of the preceding claims, wherein step (ii) comprises diluting the purified keratin prior to mixing.

19. The method of claim 18, wherein the purified keratin is diluted to ~~about~~ 0.5 mg/ml to ~~about~~ 100 mg/ml in the dialysis buffer solution.

20. The method of any one of the preceding claims, wherein step (ii) comprises mixing a substantially equal volume of diluted purified keratin with a substantially equal volume of solution of salt in an acidic buffer solution.

21. The method of any one of the preceding claims, wherein step (ii) is performed for a duration of ~~about~~ 1 minute to ~~about~~ 120 minutes.

22. The method of any one of the preceding claims, wherein the method further comprises step (ia) extracting keratin from a keratin protein source with an extraction solution to form a solubilized keratin solution, wherein step (ia) occurs before step (i).

23. The method of claim 22, wherein the keratin protein source is selected from the group consisting of hair, wool, fur, horns, hooves, beaks, feathers, and scales.

24. The method of any one of the preceding claims, wherein the solubilized keratin solution is substantially free of keratin-associated protein (KAP).

~~25. A keratin filament network comprising at least 2 Type I human hair keratins and at least 2 Type II human hair keratins.~~

~~26. The keratin filament network of claim 25, comprising at least 4 Type I human hair keratins and at least 4 Type II human hair keratins.~~

~~27. The keratin filament network of claim 25 or 26, wherein the Type I human hair keratin is selected from the group consisting of KRT31, KRT32, KRT33A, KRT33B, KRT34, KRT35, KRT36, KRT37, KRT38, KRT39 and KRT40.~~

~~28. The keratin filament network of any one of claims 25-27, wherein the Type II human hair keratin is selected from the group consisting of KRT81, KRT82, KRT83, KRT84, KRT85 and KRT86.~~

~~29. The keratin filament network of any one of claims 25-28 comprising:~~
~~6 Type I human hair keratins selected from the group consisting of KRT31, KRT32, KRT33A, KRT33B, KRT34, and KRT35; and~~

~~6 Type II human hair keratins selected from the group consisting of KRT81, KRT82, KRT83, KRT84, KRT85 and KRT86.~~

~~30. The keratin filament network of any one of claims 25-29, wherein the average filament diameter is about 4 nm to about 15 nm.~~

~~31. The keratin filament network of any one of claims 25-30, wherein the network is a mesh.~~

~~32. A keratin filament network obtained by the method of any one of claims 1-24.~~