

KERATIN-BASED FIBERS

FIELD OF INVENTION

The present invention provides a fiber comprising two or more fibrils, the two or more fibrils each comprising an anionic α -keratin intermediate filament and a cationic polymer, the cationic polymer selected from the group consisting of a cationic polysaccharide and a cationic α -keratin intermediate filament. The present invention also provides a suture and a mesh comprising the fiber of the invention, and provides a method of preparing the fiber of the invention.

BACKGROUND

The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Human hair is generally a waste material that accumulates in large quantities in landfills or is incinerated. It degrades slowly in the environment and, if burnt, cause environmental pollution. Regardless, human hair contains abundant amounts of keratins that make up approximately 80-90% of hair's total mass. In our pursuit for sustainability, keratins have emerged as interesting alternative functional materials for a wide range of applications. Human hair keratins are α -helical proteins that are abundantly expressed in the epithelia of mammals. They assemble through a hierarchical coiled-coiled process to form keratin intermediate filaments (KIF), which make up an important cytoskeletal network within epithelial cells responsible for tissue integrity and mechanotransduction. KIFs are also important structural components in appendages such as hair and nails, where they are bundled and surrounded by keratin associated proteins, which act as a matrix to hold the KIF in place to form a strong composite material. In recent decades, human hair keratins have been widely explored as a biomaterial due to their biocompatibility, biodegradability and bioactivity. As they are human-derived, there is a lower risk of interspecies disease transmission compared to animal-derived biomaterials and are more likely to circumvent religious and cultural inhibitions for use on humans. Human hair keratins can be made into various forms and some examples include films, hydrogels, fibers, scaffolds and sponges for biomedical applications including wound healing and tissue engineering.

Keratin fibers are commonly produced through electrospinning. However, electrospun keratin fibers have often been reported to have poor mechanical properties. Hence, they are often blended with synthetic polymers to improve their strength. Zhao *et al.* (*Materials Science and Engineering: C*, vol. 49, pp. 746-753, 2015) reported a human hair keratin electrospun scaffold

blended with poly-caprolactone (PCL) for bone tissue regeneration. The scaffold had poor mechanical properties with an Ultimate Tensile Strength (UTS) of 13 MPa and a Young's modulus of 15 MPa. Aluigi *et al.* (*European Polymer Journal*, vol. 44, no. 8, pp. 2465-2475, 2008) reported a wool-derived keratin electrospun scaffold blended with poly(ethylene oxide) (PEO) for cells or filters. The mechanical properties showed that the addition of increased amounts of keratin from 10% to 70% reduced the Young's modulus from 12 MPa to 7 MPa.

Thus, there is a need for alternative and/or improved fibers suitable for biomedical applications, and alternative and/or improved methods of preparing such fibers.

SUMMARY OF INVENTION

The present invention provides a fiber comprising two or more fibrils, the two or more fibrils each comprising an anionic α -keratin intermediate filament and a cationic polymer, the cationic polymer selected from the group consisting of a cationic polysaccharide and a cationic α -keratin intermediate filament.

The present invention also provides a suture comprising one or more fibers according to the present invention, and also provides a mesh comprising one or more fibers according to the present invention. The suture of the present invention may find utility in a method of surgery comprising closing a wound on a mammalian body (e.g. a human body) with the suture. The mesh of the present invention may find utility in a method of surgery comprising inserting the mesh into a mammalian body (e.g. a human body) to strengthen damaged or weakened tissues.

The present invention further provides a method of preparing the fiber of the present invention, said method comprising:

- (a) providing an aqueous solution of the anionic α -keratin intermediate filament and an aqueous solution of the cationic polymer;
- (b) contacting the aqueous solution of the anionic α -keratin intermediate filament and the aqueous solution of the cationic polymer under conditions conducive to form an interface; and
- (c) pulling a fibril formed from the anionic α -keratin intermediate filament and cationic polymer from the interface;
- (d) forming a fiber of the invention by combining two or more portions of the fibril from step (c) or combining two or more fibrils formed by repeating steps (a) to (c).

Preferred but optional features are set out in the dependent claims. Additional aspects and embodiments of the fiber, methods and uses of the present invention will be apparent from the following description, figures and claims. As can be appreciated from the foregoing and

following description, each and every feature described herein, and each and every combination of two or more such features, is included within the scope of the present disclosure provided that the features included in such a combination are not mutually inconsistent. In addition, any feature or combination of features may be specifically excluded from any embodiment.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 depicts the extraction of human hair keratin and fabrication of keratin-based fibers by Interfacial Polyelectrolyte Complexation (IPC). (a) Schematic to show how cationic α -keratin intermediate filaments (K⁺) and anionic α -keratin intermediate filaments (K⁻) are formed from two different dialysis methods. (b) Zeta potential measurements of cationic chitosan (C⁺), K⁻ and K⁺, presented as means $\pm$ standard deviation (N = 3, n = 3). (c) Viscosity measurements of C⁺, K⁻ and K⁺, presented as means $\pm$ standard deviation (N = 3, n = 3). (d) Schematic representation of how the oppositely charged polyelectrolytes (i.e. C⁺, K⁻ and K⁺) interact to form CK (i.e. fiber formed from C⁺ and K⁻) and KK (i.e. fiber formed from K⁺ and K⁻) fibers. (e) Light microscope images of a freshly pulled CK and KK fiber to show the characteristic nervation pattern seen for fibers formed by IPC (scale bar = 100 μ m).

FIG. 2 depicts the functional groups and protein secondary structure of fibers formed by IPC (i.e. CK and KK fibers) and the polyelectrolyte solutions (i.e. K⁺, K⁻ and C⁺ solutions). (a) Representative ATR-FTIR spectra showing five amide bonds of proteins and the free thiol (SH) groups in the zoomed in spectra (n = 3). (b) Deconvoluted Amide I peaks showing constituent protein secondary structures. (c) Percentage distribution of protein secondary structures based on deconvolution of Amide I peaks. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * p < 0.05 compared between sample groups for a particular secondary structure, # p < 0.05 comparing each secondary structure to β -sheets, data is presented as means $\pm$ standard deviation (N = 3, n = 3).

FIG. 3 shows Congo red stained CK and KK fibers under cross-polarized light microscope, a 2D WAXS scattering pattern, and a 1D WAXS radial intensity distribution showing the anisotropic nature of CK and KK fibers. (a) CK fiber at 0°, 45° and 90° (scale bar = 100 μ m). (b) KK fiber at 0°, 45° and 90° (scale bar = 100 μ m). Similar birefringence was obtained for n = 3 separate samples. (c) WAXS of CK fiber. (d) WAXS of KK fiber. Similar 2D WAXS scattering pattern and 1D WAXS radial intensity distribution were obtained for n = 3 separate samples.

FIG. 4 shows field emission scanning electron microscopy (FESEM) images showing the morphology of the CK and KK fibers, and the versatility of the fibers to be made into various forms. (a) Morphology of CK fiber. (b) Morphology of KK fiber. (c) Cross-section of CK fiber. (d) Cross-section of KK fiber. (e) Fibers weaved into a mesh with a 6 x 6 configuration. (f) Fiber tied into a simple knot.

FIG. 5 depicts the tensile properties of CK and KK fibers consisting of 10 fibrils. (a) Representative stress-strain curves of CK and KK fibers. (b) Young's modulus of CK and KK fibers. (c) Ultimate tensile strength (UTS) of CK and KK fibers. (d) Strain at break of CK and KK fibers. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * $p < 0.05$ compared between sample groups, data is presented as means $\pm$ standard deviation ($n = 10$). Results from one representative extraction batch are presented here. Reported trend is reproducible for all three extraction batches ($N = 3$, $n = 10$).

FIG. 6 depicts the thermal properties of CK and KK fibers and polyelectrolyte solutions (i.e. K⁺, K⁻ and C⁺ solutions). (a) Differential scanning calorimetry (DSC) thermographs showing the endothermic and exothermic peaks which indicate the thermal stability of the samples. (b) Thermogravimetric analysis (TGA) thermographs showing the various stages of weight loss which indicate the thermal decomposition of the samples. Weight (%) is denoted by black solid line while Derivative Weight (%/°C) is denoted by black dotted line. Similar DSC and TGA thermographs were obtained for $n = 3$ separate samples.

FIG. 7 depicts the evaluation of CK and KK fibers as suture materials. (a) Alternate suturing pattern consisting of CK or KK fibers (white arrows) and commercial polyamide sutures (black arrows) used to close a 1 cm incision on the dorsal skin of wild type C57BL/6J mice. H&E stained sections showing wound closure by (b) polyamide sutures, (c) CK fibers, and (d) KK fibers on Day 7 (scale bar = 100 μ m); asterisks (*) indicate the healed wound site. Black rectangles indicate the region of interest shown in the high magnification H&E images of the respective suture group: (bi) polyamide sutures, (ci) CK fiber, (di) KK fiber; with the neutrophils (black dotted arrows) and fibroblasts (black arrows) identified.

FIG. 8 depicts the subcutaneous implantation of samples under the dorsal skin of wild type C57BL/6J mice. H&E and Masson's trichrome stained sections of (a, b) polyglyconate suture, (c, d) CK fiber and (e, f) KK fiber, showing the comparative extent of immune cell recruitment and fibrotic encapsulation around the materials after 21 days (scale bar = 100 μ m); asterisks (*) indicate the original positions of the sutures (note: polyglyconate suture was lost due to histological processing). Black rectangles indicate the region of interest shown in the high

magnification H&E images of suture materials which show a smooth boundary around the (g) polyglyconate suture, suggesting minimal material degradation while (h) CK fiber and (i) KK fiber presented irregular, cavitated material boundaries, suggesting more pronounced degradation. Neutrophils (black dotted arrows) and fibroblasts (black arrows) are identified around the peripheral of all 3 materials.

FIG. 9 includes representative TEM images of KK fibers showing their morphology and depicts their fiber diameter. (a) Morphology of K+ filaments showing their network structure with elongated filaments that were entangled together (scale bar = 100 nm). (b) Morphology of K- filaments showing their short filaments and globular aggregates (scale bar = 100 nm). (c) Boxplot of K+ and K- fiber diameter. The minimum and maximum boundary lines represent the 25th and 75th percentile. The line within the box represents the median. Whiskers indicate the range within 1.5 IQR. Means comparison was done with Student's t-test; * $p < 0.05$ compared between samples groups, data is presented as means $\pm$ standard deviation ($n = 100$).

FIG. 10 depicts the optimization of polyelectrolyte concentrations. (a) Various concentrations of C+ and K- (used at 1:1 v/v ratio with respect to each other) used to produce CK fibers and the corresponding length of CK fiber produced. (b) Various concentrations of K+ and K- (used at 1:1 v/v ratio with respect to each other) used to produce KK fibers and the corresponding length of KK fiber produced. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * $p < 0.05$; data is presented as means $\pm$ standard deviation ($N = 3$, $n = 3$).

FIG. 11 depicts the optimization of volume to volume ratio of polycation (i.e. K+ or C+) to polyanion (K-). (a) Various v/v ratio of 25 mg/ml C+ and 9 mg/ml K- used to produce CK fibers and the corresponding length of CK fiber produced. (b) Various v/v ratio of 5 mg/ml K+ and 9 mg/ml K- used to produce KK fibers and the corresponding length of KK fiber produced. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * $p < 0.05$; data is presented as means $\pm$ standard deviation ($N = 3$, $n = 3$).

FIG. 12 depicts the protein concentration and pH of K+ and K- after dialysis. (a) Protein concentration of K+ and K- measured using the Bradford assay. (b) pH of K+ and K- measured after dialysis. Data is presented as means $\pm$ standard deviation ($n = 20$).

FIG. 13 depicts the reduced intensity, broadening and the shift to lower wavenumber of the Amide A peak for CK fibers compared to K-. Similar ATR-FTIR spectra was obtained for $n = 3$ separate samples.

FIG. 14 shows a Congo red stained K+ and K- film showing an isotropic birefringence and no amyloid formation. 2D WAXS scattering pattern and 1D WAXS radial intensity distribution showing the isotropic nature of K+ and K- films. (a) K+ film at 0°, 45° and 90° (scale bar = 100 μm). (b) K- film at 0°, 45° and 90° (scale bar = 100 μm). Similar birefringence was obtained for $n = 3$ separate samples. (c) WAXS of K+ film. (d) WAXS of K- film. Similar 2D WAXS scattering pattern and 1D WAXS radial intensity distribution were obtained for $n = 3$ separate samples.

FIG. 15 shows field emission scanning electron microscopy (FESEM) images showing the versatility of the fibers to be braided. Three strands of the respective CK and KK fibers were manually braided using a tweezer to form a multifilament fiber. (a) Morphology of braided CK fiber. (b) Morphology of braided KK fiber.

FIG. 16 depicts the tensile properties of CK and KK fibers consisting of 10 fibrils in replicates. (a) $N=2$ Young's modulus of CK and KK fibers. (b) $N=3$ Young's modulus of CK and KK fibers. (c) $N=2$ Ultimate tensile strength (UTS) of CK and KK fibers. (d) $N=3$ Ultimate tensile strength (UTS) of CK and KK fibers. (e) $N=2$ Strain at break of CK and KK fibers. (f) $N=3$ Strain at break of CK and KK fibers. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * $p < 0.05$ compared between sample groups, data is presented as means $\pm$ standard deviation ($n = 10$).

FIG. 17 shows the wound closure of polyamide suture and CK and KK fibers showing the view along the wound incision on Day 3. H&E and Masson's trichrome stained sections showing wound closure by (a, b) polyamide suture, (c, d) CK fiber, and (e, f) KK fiber on Day 3 (scale bar = 100 μm); asterisks (*) indicate the original positions of the sutures.

FIG. 18 shows the wound closure of polyamide suture and CK and KK fibers showing the view along the wound incision on Day 7. H&E and Masson's trichrome stained sections showing wound closure by (a, b) polyamide suture, (c, d) CK fiber and (e, f) KK fiber on Day 7 (scale bar = 100 μm); asterisks (*) indicate the healed wound site.

FIG. 19 shows the subcutaneous implantation of CK and KK fibers on Day 7. H&E and Masson's trichrome stained sections showing wound closure by (a, b) CK fiber and (c, d) KK fiber, showing the comparative extent of immune cell recruitment and fibrotic encapsulation around the materials after 7 days (scale bar = 100 μm); asterisks (*) indicate the original positions of the sutures.

FIG. 20 depicts the subcutaneous implantation of CK and KK fibers on Day 14. H&E and Masson's trichrome stained sections showing wound closure by (a, b) CK fiber, and (c, d) KK fiber, showing the comparative extent of immune cell recruitment and fibrotic encapsulation around the materials after 14 days (scale bar = 100 μ m); asterisks (*) indicate the original positions of the sutures.

FIG. 21 depicts the tensile properties of CK and KK fibers formed from 10 fibrils (i.e., 10x CK fibrils, or 10x KK fibrils): A) Young's modulus, B) UTS, C) Strain at break of the CK and KK fibers. CK1-3 represent three separate CK fiber samples, and KK1-3 represent three separate KK fiber samples. One-Way ANOVA, Tukey's Post Hoc Test, * $p < 0.05$, $n=7$, data is presented as mean $\pm$ standard deviation.

DESCRIPTION

The present invention provides a fiber comprising two or more fibrils, the two or more fibrils each comprising an anionic α -keratin intermediate filament and a cationic polymer, wherein the cationic polymer is selected from the group consisting of a cationic polysaccharide or a cationic α -keratin intermediate filament. As described herein, the present inventors have found that such fibers display surprisingly high tensile strength and biocompatibility properties. The tensile strength and biocompatibility of the fibers make them especially useful for medical applications such as sutures for wound closure, as meshes to strengthen damaged or weakened tissues in hernia repair, to promote blood clotting during surgical procedures, or as a scaffold for tissue regeneration or engineering.

In embodiments herein, the word "comprising" may be interpreted as requiring the features mentioned, but not limiting the presence of other features. Alternatively, the word "comprising" may also relate to the situation where only the components/features listed are intended to be present (e.g. the word "comprising" may be replaced by the phrases "consists of" or "consists essentially of"). It is explicitly contemplated that both the broader and narrower interpretations can be applied to all aspects and embodiments of the present invention. In other words, the word "comprising" and synonyms thereof may be replaced by the phrase "consisting of" or the phrase "consists essentially of" or synonyms thereof and *vice versa*.

The phrase, "consists essentially of" and its pseudonyms may be interpreted herein to refer to a material where minor impurities may be present. For example, the material may be greater than or equal to 90% pure, such as greater than 95% pure, such as greater than 97% pure, such as greater than 99% pure, such as greater than 99.9% pure, such as greater than 99.99% pure, such as greater than 99.999% pure, such as 100% pure.

As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a composition” includes mixtures of two or more such compositions.

Alpha-keratin is a type of fibrous structural protein found in mammalian hairs, horns, claws, nails and the epidermis layer of the skin. Generally, two α -keratin proteins characterized by alpha-helical structures assemble to form heterodimers with a coiled coil structure. These dimers align with each other to form protofilaments. The protofilaments aggregate to form protofibrils, which in turn aggregate to form an α -keratin intermediate filament. Alpha-keratin intermediate filaments typically have an average diameter of about 10 nm. The dimers, protofilaments and protofibrils that form an α -keratin intermediate filament may be held together by disulphide bonds formed between the α -keratin proteins. Alpha-keratin intermediate filaments embed within a keratin matrix formed from keratin associated proteins (KAPs) to form the structural components of hair, horns, claws, and other keratinized tissues.

Alpha-keratin intermediate filaments may be obtained from any suitable source. In certain embodiments, the α -keratin intermediate filaments are obtained from a mammalian source. For example, the α -keratin intermediate filaments may be obtained from mammalian hair, nails or skin. In certain preferred embodiments, the α -keratin intermediate filaments are derived from mammalian hair, for example, human hair.

The α -keratin intermediate filaments present in the fiber of the present invention have either a net negative charge or a net positive charge. When the α -keratin intermediate filament has a net negative charge it is referred to herein as an “anionic α -keratin intermediate filament”. When the α -keratin intermediate filament has a net positive charge it is referred to herein as a “cationic α -keratin intermediate filament”. Since α -keratin intermediate filaments typically have an isoelectric point of from about 4.5 to about 5.5, when the α -keratin intermediate filaments are in an aqueous solution with a pH below about 4.5-5.5, they have a net positive charge (i.e. they will be “cationic α -keratin intermediate filaments”). When the α -keratin intermediate filaments are in an aqueous solution with a pH above about 4.5-5.5, they have a net negative charge (i.e. they will be “anionic α -keratin intermediate filaments”).

Typically, the anionic α -keratin intermediate filaments described herein have a zeta potential of less than about -40 mV, for example from about -42 mV to about -47 mV (e.g. about -44 mV). An anionic α -keratin intermediate filament may be prepared by dialysis of a solution of α -keratin intermediate filaments against an aqueous solution with a pH of greater than about 5.5. For example, anionic α -keratin intermediate filaments may be prepared by dialysis of a solution of α -keratin intermediate filaments against deionised (DI) water to form a solution with a pH of about 6.5.

Typically, the cationic α -keratin intermediate filaments have a zeta potential of greater than about 30 mV, for example from about 33 mV to about 37 mV (e.g. about 35 mV). A cationic α -keratin intermediate filament may be prepared by dialysis of a solution of α -keratin intermediate filaments against one or more acidic solutions to provide a solution of α -keratin intermediate filaments with a pH of less than about 4.5, for example, less than about 4, less than about 3.5, or less than about 3.

The α -keratin intermediate filaments used to form the fiber of the present invention are typically used in a reduced state. That is to say that the thiol groups of the cysteine residues in the α -keratin intermediate filaments are in their reduced form, meaning they have not formed disulfide bonds with other cysteine residues. Typically, the α -keratin intermediate filaments are oxidised once the fiber is formed, such that disulfide bonds form cross-links between the α -keratin intermediate filaments in the fiber. The fiber of the present invention may be oxidised using an oxidising agent such as gaseous oxygen, for example, the fiber of the present invention may be oxidised by air drying the fiber.

The term "cationic polysaccharide" refers to a polysaccharide with a net positive charge. Suitable polysaccharides include, but are not limited to, cellulose, starch, methylated collagen, and chitosan. The cationic polysaccharide may be obtained by preparing an aqueous solution of a polysaccharide with a pH below the isoelectric point of the polysaccharide. In certain exemplary embodiments, the cationic polysaccharide is cationic chitosan. Cationic chitosan suitable for forming a fiber of the present invention may be prepared by dissolving chitosan in an acidic aqueous solution to form a solution of cationic chitosan with a pH of about 5, or less. For example, the cationic chitosan may be prepared by dissolving chitosan in acetic acid (e.g. a 0.1 M to 0.2 M (e.g. about 0.15 M) acetic acid solution). Typically, the cationic chitosan has a zeta potential of greater than about 30 mV, for example from about 33 mV to about 60 mV, about 40 mV to 60 mV, about 50 mV to 60 mV (e.g. about 57 mV).

Typically, the chitosan used to form a fiber of the invention has a molecular weight (Mw) of from about 50,000 Da to about 190,000 Da. The Mw of the chitosan referred to herein refers to the viscosity average molecular weight of the chitosan. The viscosity average molecular weight of chitosan may be determined by measuring the viscosity of the chitosan in solution and relating this to the chitosan's molecular weight using the Mark–Houwink–Sakurada (MHS) equation (see for example, Kasaai, Carbohydrate Polymers 68 (2007) 477-488, which is included herein by reference). Typically, the chitosan is about 70% to about 90% deacetylated, for example about 75% to about 85% deacetylated.

In certain embodiments, the fiber comprises at least 5 fibrils, for example, from about 5 fibrils to 100 fibrils, 5 fibrils to 50 fibrils, 5 fibrils to 40 fibrils, 5 fibrils to 30 fibrils, 5 fibrils to 20 fibrils,

5 fibrils to 10 fibrils. For example, the fiber may comprise from 10 fibrils, 30 fibrils or 50 fibrils. The present inventors have found that when the fiber of the present invention is formed from at least 5 fibrils (e.g. about 10 fibrils), the fiber displays tensile strength and flexibility that makes it surprisingly useful as a suture or mesh for biomedical applications.

In certain exemplary embodiments, the fiber has a cross-sectional diameter of from about 70 μm to about 200 μm , for example from about 100 μm to 200 μm , about 125 μm to 200 μm , about 150 μm to 200 μm . Typically, when the fiber is formed from about 10 fibrils, it has a cross-sectional diameter of from about 70 μm to about 200 μm , for example, from about 150 μm to about 200 μm (e.g. from 150 μm to 199 μm).

Typically, the fiber has a homogenous cross-section. That is to say that the individual fibrils that form the fiber cannot be distinguished from each other by microscopy analysis (e.g. by FESEM) of the cross-section of the fiber.

Typically, the fiber of the present invention has a length of from about 1 cm to about 50 cm, for example, from about 1 cm to about 45 cm, or from about 5 cm to about 45 cm. The length of the fiber of the invention may be easily adjusted by one skilled in the art to a size suitable for its intended use.

In certain embodiments, the α -keratin intermediate filaments are each substantially free from KAPs, for example comprise less than 10 wt% KAPs (e.g. less than 5 wt%, less than 2 wt%, less than 1 wt% KAPs). The α -keratin intermediate filaments may also be substantially free from lipids, such as fatty acids (e.g. myristic acid, palmitic acid, and stearic acid), wax esters (myristyl palmitate, palmityl palmitate, and stearyl palmitate), squalene, cholesterol, 18-methyl eicosanoic acid (18-MEA), ceramides, hydrocarbons, and triglycerides. For example the α -keratin intermediate filaments may comprise less than 10 wt% lipids (e.g. less than 5 wt%, less than 2 wt%, less than 1 wt% lipids).

As disclosed herein, the fiber of the present invention displays surprisingly useful tensile strength and flexibility. The tensile strength and flexibility of the fiber of the invention may be quantified by determining one or more of the Young's modulus, Ultimate Tensile Strength (UTS), strain-at-break, and Maximum (Max) Load of the fiber.

For the avoidance of doubt, Young's modulus is a measure of the stiffness of a material. It is a quantification of the ability of a material to withstand deformation under tensile stress along its length. Young's modulus is defined as the ratio of stress (force per unit area) applied to a material to the resulting strain (relative deformation) in the direction of the applied stress, within the region of elastic deformation. It may be expressed in units of pressure (pascal, Pa, for example megapascal (MPa) or gigapascal (GPa)) or in equivalent units, such as pounds per square inch (psi). The UTS of a material is a measure of the maximum stress that the material

can withstand before breaking. UTS is typically expressed in units of pressure (pascal, Pa, for example megapascal (MPa) or gigapascal (GPa)) or in equivalent units, such as pounds per square inch (psi). The “strain-at-break” of a fiber is a measure of the extent to which the fiber of the present invention can be stretched at the point of break. Max Load is a measure of the maximum force that a fiber of the present invention can withstand before it breaks. The maximum load may be measured in Newtons (N).

Specific methods for determining the Young's modulus, UTS, strain-at-break, and Max Load of the fiber of the present invention are disclosed in the Examples section herein.

In certain preferred embodiments, the fiber of the present invention displays one or more of the following:

- i) a Young's modulus of greater than or equal to about 0.5 GPa, for example, from about 0.5 GPa to about 2.5 GPa (e.g. about 2 GPa);
- ii) a UTS of greater than or equal to about 2.5 MPa, for example, greater than about 10 MPa, about 20 MPa, about 30 MPa, or about 40 MPa, for example, the fiber may have a UTS from about 10 MPa to about 50 MPa (e.g. about 30 MPa);
- iii) a strain-at-break of greater than about 10%, for example, greater than about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 95%;
and
- iv) a Max Load of greater than or equal to about 0.3 N, for example, from about 0.3 N to about 3.5 N.

In certain exemplary embodiments, the two or more fibrils of the fiber of the present invention each comprise an anionic α -keratin intermediate filament and a cationic polysaccharide. For example, the two or more fibrils may each comprise anionic α -keratin intermediate filaments and cationic chitosan. Such fibers of the present invention may be referred to herein as CK fibers. The present inventors have demonstrated that the CK fiber of the present invention displays tensile strength and flexibility that make it especially useful as a suture or mesh for biomedical applications. For example, as described in the Examples section herein, CK fibers of the present invention comprising 10 fibrils were demonstrated to have a Young's modulus of 2.05 ± 0.2 GPa, UTS of 32.12 ± 5.53 GPa, a strain-to-break of $16.08 \pm 6.44\%$, and max load of 0.46 ± 0.15 N. The present inventors have also demonstrated that the CK fibers can be readily weaved to form a mesh, and can be readily knotted, or braided.

Each fibril of the CK fiber may comprise the cationic chitosan and anionic α -keratin intermediate filaments at a weight ratio of cationic chitosan to anionic α -keratin intermediate filament of from about 1 to about 5, for example, about 1 to about 3.5 (e.g. about 2.8), or about 1.8 to about 2.2.

In certain embodiments, the fiber of the present invention consists of two or more fibrils, wherein each of the two or more fibrils comprises (or consists) of an anionic α -keratin intermediate filament and a cationic polysaccharide (e.g. cationic chitosan).

In certain other exemplary embodiments, the two or more fibrils of the fiber of the present invention each comprise an anionic α -keratin intermediate filament and a cationic α -keratin intermediate filament. Such fibers of the present invention may be referred to herein as KK fibers. The present inventors have demonstrated that the KK fiber of the present invention displays tensile strength and flexibility that make it especially useful as a suture or mesh for biomedical applications. For example, as described in the Examples section herein, KK fibers of the present invention formed from 10 fibrils were demonstrated to have a mean Young's modulus of 0.88 ± 0.19 GPa, UTS of 28.56 ± 6.51 GPa, a strain-to-break of $96.63\% \pm 17.24\%$, and max load of 0.33 ± 0.08 N. The present inventors have also demonstrated that the KK fibers can be readily weaved to form a mesh, and can be knotted, or braided.

Each fibril of the KK fiber may comprise the cationic α -keratin intermediate filament and anionic α -keratin intermediate filament at a weight ratio of cationic α -keratin intermediate filament to anionic α -keratin intermediate filament of from about 0.1 to about 1, for example, from about 0.1 to about 0.8, or about 0.4 to about 0.7 (e.g. about 0.6).

In certain embodiments, the fiber of the present invention consists of two or more fibrils, wherein each of the two or more fibrils comprises (or consists) of an anionic α -keratin intermediate filament and a cationic α -keratin intermediate filament.

In certain other embodiments, the fiber of the present invention comprises at least one fibril comprising an anionic α -keratin intermediate filament and a cationic α -keratin intermediate filament, and at least one fibril comprising an anionic α -keratin intermediate filament and a cationic polysaccharide. For example, the fiber of the present invention may consist of at least one fibril comprising (or consisting) of an anionic α -keratin intermediate filament and a cationic α -keratin intermediate filament, and at least one fibril comprising (or consisting) of an anionic α -keratin intermediate filament and a cationic polysaccharide.

For the avoidance of doubt, features of the fiber of the invention disclosed herein, such as the diameter, length, tensile strength and flexibility of the fiber, refer to the fiber when in a dried form (e.g. a fiber that has been air-dried at room temperature, as described in Example 1 herein), unless stated otherwise.

Utility:

The present inventors have demonstrated in an *in vivo* wound closure experiment using mice that the fibers of the present invention are flexible and can withstand knotting when used as a suture to close a wound. The fibers were also demonstrated in the *in vivo* wound closure

experiment to facilitate successful wound closure over 7 days, with no observable acute inflammation. Furthermore, the fibers were found to show some signs of degradation 21 days after being implanted into a mouse, and did not evoke an excessive host tissue response, thus demonstrating that the fibers were well tolerated and could be used as a suture material.

Thus, the present invention also provides a suture comprising one or more fibers of the present invention. Typically, the suture of the present invention is formed from a fiber disclosed herein comprising at least 5 fibrils, for example at least 10 fibrils, at least 30 fibrils or at least 50 fibrils. In certain exemplary embodiments, the suture is formed from a fiber disclosed herein that comprises 10 fibrils. Typically, when the fiber is formed from 10 fibrils, it has a cross-sectional diameter of from about 70 μm to about 200 μm , for example, about 150 μm to about 200 μm (e.g. from 150 μm to 199 μm). Thus, the suture of the present invention may have a U.S. PHARMACOPEIA (USP) size of 5-0 (i.e. it may have a cross-sectional diameter from about 150 μm to about 199 μm).

The biocompatibility and tensile strength of the fiber also makes it an attractive material for other surgical applications. For example, when the fiber of the present invention is in the form of a mesh, it may find use as a reinforcing material to strengthen damaged or weakened tissues in hernia repair or as a material to promote blood clotting during surgical procedures. Additionally, or alternatively, a mesh formed from the fiber of the invention may find use as a scaffold for tissue regeneration and engineering. Thus, the present invention also provides a mesh comprising one or more fibers of the present invention. Typically, the mesh is formed from a plurality of fibers of the present invention. For example, the mesh may be formed from a plurality of CK fibers of the invention, a plurality of KK fibers of the invention, or a combination of CK fibers and KK fibers of the invention.

As evident from the disclosure herein, the fiber of the present invention may find utility in a method of surgery comprising closing a wound with the suture of the present invention. For example, a method comprising closing a wound by forming stitches with the suture which then act to close the edges of the wound and facilitate healing of the wound. The wound may be on the body of a mammal, for example on the body of a human. The fiber of the present invention may also find utility in a method of surgery comprising inserting a mesh comprising a plurality of fibers of the present invention into a mammalian body (e.g. a human body) to strengthen damaged or weakened tissues. Such a method may, for example, be employed to repair a hernia in the mammalian body.

The suture and mesh of the present invention may be sterilised. For example, the suture and mesh may be sterilised using common techniques used for sterilisation of biomaterials, for

example, the suture and mesh may be sterilised using radiation (e.g. ultraviolet or gamma), ethanol, or ethylene oxide.

Method of manufacture:

As described herein, the present inventors have demonstrated that keratin-based fibers with surprisingly high tensile strength and biocompatibility properties can be produced using Interfacial Polyelectrolyte Complexation (IPC). IPC can be used to form fibers by the complexation of oppositely charged polyelectrolytes at an interface formed between the two polyelectrolytes. At this interface, small fragments of nanofibrils grow and elongate into longer fibers as the material is drawn from the interface. Thus, in certain embodiments, the fibers of the present invention are formed by IPC. Accordingly, the present invention also provides a method of preparing the fiber of the present invention by IPC, said method comprising:

- (a) providing an aqueous solution of the anionic α -keratin intermediate filament and an aqueous solution of the cationic polymer;
- (b) contacting the aqueous solution of the anionic α -keratin intermediate filament and the aqueous solution of the cationic polymer under conditions conducive to form an interface; and
- (c) pulling a fibril formed from the anionic α -keratin intermediate filament and the cationic polymer from the interface;
- (d) forming the fiber according to the invention by combining two or more portions of the fibril from step (c) or combining two or more fibrils formed by repeating steps (a) to (c).

In certain embodiments, step (a) of the method further comprises providing a sample of mammalian hair, followed by extracting lipids from the sample using an organic solvent and extracting KAPs from the sample using a denaturing agent, thereby providing a sample of α -keratin intermediate filaments, optionally wherein the sample of α -keratin intermediate filaments is an aqueous solution of α -keratin intermediate filaments. Any suitable organic solvent may be used to extract lipids from the mammalian hair, and any suitable denaturing agent may be used to extract KAPs from the mammalian hair. Examples of suitable organic solvents include chloroform, acetone, methanol, diethyl ether, and mixtures thereof. Examples of suitable denaturing agents include urea, guanidine hydrochloride, sodium dodecyl sulfate, and mixtures thereof. In certain exemplary embodiments, the lipids are extracted from mammalian hair using a mixture of chloroform and methanol (e.g. a 2:1 (v/v) ratio of chloroform and methanol), and KAPs are extracted from the mammalian hair using urea (e.g. 8 M urea in an aqueous buffer).

In certain embodiments, step (a) of the method further comprises reducing the sample of α -keratin intermediate filaments with a reducing agent. By treating the α -keratin intermediate

filaments with a reducing agent, it is possible to convert thiol groups of the cysteine residues in the α -keratin intermediate filaments to their reduced form. Any suitable reducing agent may be used to reduce the α -keratin intermediate filaments, for example, the reducing agent may be dithiothreitol (DTT), tris(2-carboxyethyl)phosphine (TCEP), or β -mercaptoethanol (β -ME). Typically, the reducing agent used is DTT.

In certain embodiments, step (a) of the method further comprises providing a sample of α -keratin intermediate filaments in an aqueous solution, followed by exchanging the aqueous solution with deionised water, thereby providing an aqueous solution of anionic α -keratin intermediate filaments. This may be achieved by dialysis of the aqueous solution of α -keratin intermediate filaments, obtained following extraction of lipids and KAPs, against deionised water. Typically, the aqueous solution of anionic α -keratin intermediate filaments contains anionic α -keratin intermediate filaments at a concentration of from about 4 mg/ml to about 15 mg/ml, for example, from about 4 mg/ml to about 10 mg/ml, about 5 mg/ml, about 7 mg/ml or about 9 mg/ml.

In embodiments wherein the cationic polymer is a cationic α -keratin intermediate filament, step (a) of the method may further comprise providing a sample of α -keratin intermediate filaments in an aqueous solution obtained following extraction of lipids and KAPs, followed by reducing the pH of the aqueous solution to a pH below about 4.5 (e.g. below about 4, below about 3.5, or below about 3), thereby providing an aqueous solution of cationic α -keratin intermediate filaments. The step of reducing the pH of the aqueous solution of α -keratin intermediate filaments may be achieved by dialysis of the aqueous solution against an acidic buffer, such as a citric acid buffer. The dialysis may be performed in a stepwise manner with a decreasing concentration of denaturing agent in the acidic buffer (e.g. a decreasing concentration of urea from about 8 M to about 0 M). Typically, a suitable reducing agent (e.g. DTT) is present in the aqueous solution of α -keratin intermediate filament during the dialysis process.

Typically, the aqueous solution of cationic α -keratin intermediate filaments in step (a) contains the cationic α -keratin intermediate filaments at a concentration of from about 1 mg/ml to about 12 mg/ml, for example from about 1 mg/ml to about 6 mg/ml, about 1 mg/ml to about 5 mg/ml, or from about 4 mg/ml to about 6 mg/ml. For example, the aqueous solution of cationic α -keratin intermediate filaments may contain cationic α -keratin intermediate filaments at a concentration of about 1 mg/ml, about 2 mg/ml, about 3 mg/ml, about 4 mg/ml, or about 5 mg/ml.

In certain embodiments, wherein the cationic polymer is a cationic α -keratin intermediate filament, the aqueous solution of cationic α -keratin intermediate filaments contains the cationic α -keratin intermediate filaments at a concentration of from about 1 mg/ml to about 5 mg/ml,

and the aqueous solution of anionic α -keratin intermediate filaments contains anionic α -keratin intermediate filaments at a concentration of about 9 mg/ml.

In embodiments wherein the cationic polymer is a cationic polysaccharide (e.g. cationic chitosan), step (a) of the method further comprises providing a sample of the polysaccharide, followed by dissolving the polysaccharide in an acidic aqueous solution. In this step, the pH of the acidic aqueous solution is below the isoelectric point of the polysaccharide, such that when the polysaccharide is dissolved in the acidic aqueous solution, it has a net positive charge (i.e. it is a cationic polysaccharide). For example, in embodiments wherein the cationic polymer is cationic chitosan, this may be achieved, for example, by dissolving the chitosan in an aqueous solution of acetic acid (e.g. a 0.1 M to 0.2 M (e.g. about 0.15 M) acetic acid solution) followed by dilution with water. In such embodiments, the aqueous solution of cationic polymer may contain the cationic chitosan at a concentration of from about 10 mg/ml to about 30 mg/ml, for example from about 10 mg/ml to about 25 mg/ml, for example, about 15 mg/ml or about 25 mg/ml. In certain embodiments, wherein the cationic polymer is cationic chitosan, the aqueous solution of cationic polymer contains the cationic chitosan at a concentration of from 10 mg/ml to about 30 mg/ml (e.g. about 15 mg/ml or about 25 mg/ml), and the aqueous solution of anionic α -keratin intermediate filaments contains anionic α -keratin intermediate filaments at a concentration of about 9 mg/ml.

Typically, step (b) of the method comprises contacting the aqueous solution of the cationic polymer and the aqueous solution of the anionic α -keratin intermediate filament at a volume to volume ratio of from about 0.1 to about 6 (i.e. volume of aqueous solution of the cationic polymer to the volume of aqueous solution of the anionic α -keratin intermediate filament). For example, from about 0.2 to about 5 (e.g. about 0.2, about 0.5, about 1, about 2, about 5). In certain preferred embodiments, step (b) comprises contacting the aqueous solution of the cationic polymer and the aqueous solution of the anionic α -keratin intermediate filament at a volume to volume ratio of about 1.

In certain embodiments when the cationic polymer is cationic chitosan, the aqueous solution of cationic chitosan has a cationic chitosan concentration of about 25 mg/ml, the aqueous solution of anionic α -keratin intermediate filaments has an anionic α -keratin intermediate filament concentration of about 9 mg/ml, and the aqueous solution of the cationic chitosan and the aqueous solution of the anionic α -keratin intermediate filament are contacted to form an interface at a volume to volume ratio of about 1 (i.e. volume of aqueous solution of the cationic polymer to the volume of aqueous solution of the anionic α -keratin intermediate filament).

In certain other embodiments when the cationic polymer is a cationic α -keratin intermediate filament, the aqueous solution of cationic α -keratin intermediate filaments has a cationic α -

keratin intermediate filament concentration of about 5 mg/ml, the aqueous solution of anionic α -keratin intermediate filaments has an anionic α -keratin intermediate filaments concentration of about 9 mg/ml, and the aqueous solution of the cationic α -keratin intermediate filaments and the aqueous solution of the anionic α -keratin intermediate filament are contacted to form an interface at a volume to volume ratio of about 1 (i.e. volume of aqueous solution of the cationic α -keratin intermediate filaments to the volume of aqueous solution of the anionic α -keratin intermediate filament).

Step (c) of the method can be achieved using any suitable method for pulling a fibril from the interface formed between the aqueous solution of the anionic α -keratin intermediate filament and the aqueous solution of the cationic polymer. For example, a fibril may be drawn from the interface manually using a tweezer, or a similar hand tool. Alternatively, the step of drawing a fibril from the interface may be automated.

In certain embodiments, step (d) of the method comprises spooling the fibril formed at the interface onto itself, thereby combining two or more portions of the fibril to form the fiber. Step (d) may be repeated as necessary to form a fiber of the invention that has the desired length and/or cross-sectional diameter.

In certain embodiments, the method further comprises a step following step (d) of oxidising the fiber of the present invention. As described herein, the step of oxidising the fiber results in the formation of disulphide bonds between cysteine residues present in the α -keratin intermediate filaments that form the fiber. The fiber may be oxidised using an oxidising agent such as gaseous oxygen, for example, the fiber of the present invention may be oxidised by air drying the fiber.

The fiber of the present invention may be subjected to sterilisation before use, for example, the fibre may be subjected to radiation (e.g. ultraviolet or gamma), ethanol, or ethylene oxide sterilisation.

Further aspects and embodiments of the invention will now be discussed by reference to the following non-limiting examples.

EXAMPLES

In Vivo Studies

All experiments were conducted in accordance with the guidelines of the National Advisory Committee for Laboratory Animal Research and approved by the Institutional Animal Care and Use Committee of Nanyang Technological University, Singapore (NTU-IACUC).

Statistical Analysis

All quantitative data were generated using sample sizes of $n = 3$ unless otherwise stated, and presented as means $\pm$ standard deviation (SD). For comparison of means between more than 2 sample groups, One-Way Analysis of Variance (ANOVA) with Tukey's post hoc test was carried out using OriginPro, where $p < 0.05$ was considered statistically significant.

Example 1: Fabrication of CK and KK fibers by IPC

Human hair keratin extraction

Human hair keratins were extracted according to a previously established procedure (Tan *et al.*, *Materials Today Communications*, vol. 30, no. 103049, pp. 1-12, 2022). In brief, human hair was collected from local hair salons and thoroughly cleaned using soap and ethanol (95%, Aik Moh). Subsequently, the hair was delipidized in a 2:1 v/v ratio of chloroform (Fisher Scientific) and methanol (Aik Moh). Delipidized hair were soaked in the KAP extraction solution which consisted of 25 mM Tris-HCl (Sigma-Aldrich) at pH 9.5, 8 M urea (Gold Biotechnology), absolute ethanol (Sigma-Aldrich) and 200 mM DTT (Gold Biotechnology) at 50 °C for 72 hours. DI water was used to wash the "KAP-free" hair obtained after filtration before they were left in the fume hood to air-dry. "KAP-free" hair was soaked in the keratin extraction solution which consisted of 25 mM Tris-HCl at pH 8.5, 5 M urea, 200 mM DTT as well as 2.6 M thiourea (Sigma-Aldrich) at 50 °C for 24 hours. Subsequently, the keratin filtrate obtained was centrifuged to remove debris at a speed of 5000 rpm for 20 minutes.

Step-down dialysis to obtain a positively charged keratin solution (K+)

The step-down dialysis process was previously reported by Lai *et al.* (*ACS Biomaterials Science & Engineering*, vol. 7, no. 1, pp. 83-89, 2020) and briefly depicted in FIG 1(a). The keratin filtrate obtained after centrifugation was poured into SnakeSkin Dialysis Tubings (10K MWCO, Thermo Scientific) and dialysed against a 2.5 mM citric acid buffer solution (Sigma-Aldrich) at pH 2.96, with 8 M urea, overnight. Subsequently, dialysis in decreasing concentrations of urea from 4 M (3 hours) to 2 M (3 hours) and finally in 0 M urea (overnight) was carried out. 1 mM DTT was added to all four dialysis steps and dialysis was conducted at room temperature.

Dialysis in DI water to obtain a negatively charged keratin solution (K-)

The keratin filtrate obtained after centrifugation was poured into SnakeSkin Dialysis Tubings (10K MWCO, Thermo Scientific) and dialysed against DI water at room temperature to remove urea and DTT, to obtain K- (FIG. 1(a)).

Bradford assay

Bradford assay was used to measure the protein concentration of K⁺ and K⁻ after dialysis. Proteins bind stably to Coomassie Brilliant Blue (Bio-Rad) G-250 dye. Quick Start Bradford (Bio-Rad) 1x Dye Reagent is removed from the 4 °C fridge and left in room temperature to cool. K⁺ and K⁻ were diluted and measured against BCA standards (Thermo Scientific) with a known concentration range of 125 to 2000 µg/ml. Quick Start Bradford 1x Dye Reagent (250 µl) was added to each sample (5 µl) and left to react at room temperature for 5 minutes. The absorbance was read at a wavelength of 595 nm with the microplate reader (Infinite M200, Tecan Lifesciences).

Synthesis of a positively charged chitosan solution (C⁺)

Chitosan (low molecular weight, Sigma-Aldrich) was dissolved at a 5% weight per volume ratio in 0.15 M acetic acid (≥99%, Sigma-Aldrich) and diluted with an equal volume of DI water. The final concentration of C⁺ was 25 mg/ml.

Fabrication of Keratin-based fibers by IPC

The concentration of K⁺ was adjusted to 5 mg/ml with 2.5 mM citric acid (pH 2.96) and the concentration of K⁻ was adjusted to 9 mg/ml with DI water. The concentration of C⁺ was 25 mg/ml. Two keratin-based fibers were prepared by IPC, namely CK, which was formed from the complexation of C⁺ and K⁻ and KK, which was formed from the complexation of K⁺ and K⁻. The polyelectrolyte solutions were extruded using a syringe pump (KDS 100 Legacy Syringe Pump, KD Scientific) at a continuous rate of 5 ml/hr. CK fibers were produced in a 1:1 v/v ratio of C⁺ and K⁻. KK fibers were produced in a 1:1 v/v ratio of K⁺ and K⁻. Once the oppositely charged polyelectrolyte solutions were brought into contact with a tweezer, a fibril was drawn vertically upwards from the complexation interface. This fibril was wound onto a Teflon rod of 1.5 cm diameter using a customized spooling machine (Smart Memories Pte. Ltd.), at a draw speed of 0.39 cm/s. A bundled fiber consisting of 10 fibrils was formed by spooling a fibril repeatedly onto itself until the desired number of rounds was reached. Freshly drawn fibers were left to air-dry overnight in a fume hood at room temperature.

Fabrication of K⁺, K⁻ and C⁺ film

The three polyelectrolyte solutions (K⁺, K⁻ and C⁺) were cast into films and used as control samples. Aliquots of 1 ml of each polyelectrolyte solution were poured separately into circular polypropylene moulds (caps of 50 ml centrifuge tubes) and left to dry in a 60 °C oven to form a film.

Zeta potential

The zeta potential of the three polyelectrolyte solutions were determined with a zeta potential analyser (Zetasizer Nano ZS, Malvern Panalytical). K+, K- and C+ were diluted in 2.5 mM citric acid at pH 2.96, DI water as well as 0.15 M acetic acid respectively. The zeta potentials of a series of dilutions for each polyelectrolyte solution were measured and averaged.

Rheometer

The viscosities of the three polyelectrolyte solutions (K+, K- and C+) were measured with a rheometer (Anton Paar MCR 501). A CP25-1 spindle with a 25 mm diameter probe and 2° angle of cone was used. The viscosity of the three polyelectrolyte solutions were measured for a linear increase in shear rate for a range of 0.01-50 s⁻¹.

Results and Discussion

FIG. 9(a) and (b) are representative TEM images of the K+ and K- intermediate filaments prepared using the method described in Example 1. Specifically, FIG. 9(a) shows the morphology of K+ filaments showing their network structure with elongated filaments that were entangled together (scale bar = 100 nm), and FIG. 9(b) shows the morphology of K- filaments showing their short filaments and globular aggregates (scale bar = 100 nm). FIG. 9(c) is a boxplot of K+ and K- filaments diameter. The minimum and maximum boundary lines represent the 25th and 75th percentile. The line within the box represents the median. Whiskers indicate the range within 1.5 IQR. Means comparison was done with Student's t-test; * p < 0.05 compared between samples groups, data is presented as means ± standard deviation (n = 100).

By combining droplets of K+/K- and C+/K- in a classical IPC fiber drawing setup, fibers of defined morphology and consistency were obtained. This was possible based on the anticipated mechanisms of interaction between the materials used (FIG. 1(d)). The CK fiber was formed primarily through the electrostatic interaction of amine (NH₃⁺) groups on C+ and the carboxyl (COO⁻) groups on K-. Hydrogen bonding between the hydroxyl (OH) and amine (NH₂) groups within the system further stabilized the fibers. The KK fiber was formed through the electrostatic interaction of amine (NH₃⁺) groups on K+ and the carboxyl (COO⁻) groups on K-. Hydrogen bonding between the carboxylic acid (COOH) groups on K+ and amine (NH₂) groups on K- was also plausible for KK fibers. In addition, disulfide bonding between thiol groups (SH) on K+ and K-, together with hydrogen bonding, allowed the assembly of the fibers. Both CK and KK fibers developed exhibit the characteristic nervation pattern of fibers produced through the IPC process (FIG. 1(e)). It was observed that the CK fibers produced were more stable and continuous compared to the KK fibers, resulting from the larger zeta

potential difference between C+ and K- and the higher viscosity of C+, allowing more chain entanglement and stronger interactions to occur.

FIG. 12 depicts the protein concentration and pH of the K+ and K- filaments after the dialysis step, as described in Example 1. The pH of K+ after step-down dialysis was 3.3 which resulted in a positively charged solution as confirmed from the zeta potential measurement of 35.11 ± 0.92 mV (FIG. 1(b)). The pH of K- after dialysis in DI water was 6.5 which resulted in a negatively charged solution correlating to the zeta potential measurement of -44.44 ± 1.59 mV. Chitosan was dissolved in 0.15 M acetic acid which protonated the amino group of glucosamine, hence C+ had a positive charge of 56.58 ± 5.99 mV. The three polyelectrolyte solutions showed a shear thinning behaviour as viscosity decreased with increased shear rates (FIG. 1(c)), consistent with the literature (Islam *et al.*, *Carbohydrate Polymers*, vol. 275, no. 118682, pp. 1-12, 2022).

Example 2: Functional groups and protein secondary structures

Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR (Frontier, PerkinElmer) was used in the determination of functional groups in the CK and KK fibers and the three polyelectrolyte solution films (K+, K- and C+). The CK and KK fibers were air dried prior to testing. Each sample was pressed against the ATR crystal and a constant pressure of 90% was applied. The absorbance spectra were collected at a resolution of 4 cm^{-1} for 32 scans and for a $4000\text{--}600\text{ cm}^{-1}$ wavenumber range. Protein secondary structures present in the K+ and K- filaments and the CK and KK fibers were determined with the OriginPro software through Amide I peak deconvolution at a wavenumber range of $1600\text{--}1700\text{ cm}^{-1}$.

Results and Discussion

ATR-FTIR spectra of all samples containing keratins (K+, K-, CK and KK) showed the presence of all five amide bond absorbance peaks (Amide A, B, I, II and III) (FIG. 2(a)). The spectra for C+ showed the Amide I and Amide II peaks and was in good agreement with other reported papers (Derkach *et al.*, *Polymers*, vol. 12, no. 2, pp. 1-14, 2020 and Ferrero *et al.*, *Journal of Nanoscience and Nanotechnology*, vol. 12, no. 6, pp. 4803-4810, 2012). Amide A and Amide B peaks represented NH stretching and occurred at 3300 cm^{-1} and 3100 cm^{-1} correspondingly. Amide I peak represented C=O stretching and occurred at $1600\text{ to }1700\text{ cm}^{-1}$. Amide II and Amide III peaks occurred at $1480\text{ to }1575\text{ cm}^{-1}$ and $1229\text{ to }1301\text{ cm}^{-1}$, representing CN stretching and NH bending, respectively.

From the ATR-FTIR spectra of CK fibers, 4 peaks located at 2875, 1450, 1073 and 699 cm^{-1} had higher intensities compared to the spectra for pure K-. These 4 peaks are characteristic of chitosan and are present in the C+ spectrum. Hence this shows the successful incorporation of C+ in the CK fiber. The CH stretching peak occurred at 2875 cm^{-1} . The 1450 cm^{-1} peak corresponded to CH bending of CH_2OH . The 1073 cm^{-1} peak represented free amino group of chitosan at the glucosamine C_2 position. The 699 cm^{-1} peak was indicative of out-of-plane NH bending. The OH stretching peak of C+ occurred at 3269 cm^{-1} , overlapping with the Amide A peak of K- which occurred at 3282 cm^{-1} . The reduced intensity, broadening and the shift to lower wavenumber of the Amide A peak for CK fibers compared to K- suggested hydrogen bonding between the OH group in C+ as well as the NH_2 group in K- (FIG. 13).

The human hair keratins had high relative cysteine amounts, resulting in the free thiol (SH) groups registering in the FTIR spectra by a peak between 2550 cm^{-1} to 2560 cm^{-1} . This was shown as a weak peak in K+ and K- spectra at 2562 cm^{-1} and 2558 cm^{-1} , respectively, and at 2566 cm^{-1} in the CK fiber spectrum (FIG. 2(a), zoom in panels). However, this SH peak was not observed in the KK fiber spectra, suggesting disulfide bonding between the SH groups of K+ and K-. Furthermore, no SH peak was observed in C+.

The secondary structure band assignments and distribution (FIG. 2(b)) were determined from Amide I peak deconvolution made in accordance to Yang *et al.* (*Nature Protocols*, vol. 10, no. 3, pp. 382-396, 2015) (FIG. 2(c)). The secondary structure that dominated the CK and KK fibers, and polyelectrolytes solutions K+ and K-, was β -sheet. The proportions of β -sheets present in these samples were significantly higher in comparison to all other secondary structures. This was in agreement to Tan *et al.* (*Materials Today Communications*, vol. 30, no. 103049, pp. 1-12, 2022) where human hair keratin films were found to consist of majority β -sheets as well. Natively, human hair keratins are mostly made up of α -helices. However, during the formation of fibers, α -helix to β -sheet transition occurred, a phenomenon that is known to happen in keratins when mechanically induced. The presence of a significant proportion of 3_{10} helix, which acts as an intermediary conformation for the transition of α -helices into β -sheets, supports this.

Example 3: Amyloid Fibers

Congo Red Staining

The CK and KK fibers as well as the K+ and K- films were stained with Congo red (Sigma-Aldrich) adapted from a previously reported protocol by Fu *et al.* (*Nanoscale*, vol. 9, no. 35, pp. 12908-12915, 2017). Briefly, the fibers and films were stained with 1% Congo red in 10%

ethanol for 1 hour 30 minutes. After which, the Congo red stained samples were immersed in Milli-Q water for 30 minutes. The samples were transferred onto glass slides and left to air-dry in the fume hood before they were sealed with Cytoseal 60 (Thermo Scientific). The Congo red stained samples were visualized under cross-polarized light microscope (Axio Scope.A1, Zeiss).

Wide-Angle X-Ray Scattering (WAXS)

The CK and KK fibers as well as the K⁺ and K⁻ films were evaluated for their ability to form a cross- β diffraction pattern using WAXS (Nano-inXider, Xenocs). The CK and KK fibers were mounted parallel to the beam path. The X-ray source is Cu-K α and each scattering pattern was acquired at medium resolution. An 800 μ m beam diameter was directed onto the sample for 150 seconds for each psi rotation (0°, 18°, 36°, 54°, 72°, 90°, 108°, 126°, 144°, 162°, 180°). The Foxtrot 3.2.7 software was used to obtain the 2D WAXS scattering pattern and 1D WAXS radial intensity distribution for each sample.

Results and Discussion

A majority proportion of β -sheet structure present in CK and KK fibers, coupled with the highly aligned nature seen for fibers formed by IPC suggested the possibility of amyloid fiber formation in these fibers. Congo red staining and WAXS were hence used to evaluate the formation of amyloid fibers.

Congo red staining is a preliminary technique to show amyloid fiber formation as red dye stains stacked β -sheet structures. Under cross-polarized light, the Congo red stained CK (FIG. 3(a)) and KK fibers (FIG. 3(b)) exhibited green birefringence against a dark background which was indicative of amyloid fibers. This green birefringence was similar to Congo red stained artificial hagfish protein fibers which was similarly indicative of the β -sheet orientation of amyloid-like fibers. In addition, the fibers were also anisotropic as the birefringence intensity was maximum at 45°. As controls, Congo red stained K⁺ (FIG. 14(a)) and K⁻ (FIG. 14(b)) films showed an isotropic behaviour under cross-polarized light as they retained the red birefringence from 0° to 45°. This suggested the random nature of the fibers and a lack of molecular alignment when K⁺ and K⁻ samples were independently cast into films. It also showed the inability of K⁺ and K⁻ to form amyloid fibers independently.

WAXS was used to accurately identify the protein conformation of the CK and KK fibers and the individual polyelectrolyte solutions. The 2D WAXS scattering patterns of CK (FIG. 3(c)) and KK (FIG. 3(d)) fibers were anisotropic, which suggested molecular alignment within the fibers. This was in contrast to the scattering pattern of the controls. K⁺ (FIG. 14(c)) and K⁻

(FIG. 14(d)) films showed the presence of clear concentric circles in their WAXS scattering patterns which indicated their isotropic behaviour and further proved the lack of molecular orientation in the cast films. Therefore, these results collectively showed that the IPC process facilitated molecular interactions and alignment, producing CK and KK fibers that were anisotropic.

Furthermore, the CK and KK fibers produced a cross- β diffraction pattern which was indicative of amyloid fibers. Based on the 1D WAXS radial intensity distribution, two diffraction arcs were observed for CK and KK fibers. The first diffraction arc at $d = 0.96$ nm was weak and indicated the intersheet spacing. The second diffraction arc at $d = 0.46$ nm was brighter and stronger which indicated the interstrand spacing within the cross- β structure of amyloids. In addition, it also indicated that the β -strands were assembled at right angles to the CK and KK fibers and therefore hydrogen bonding was parallel to the fiber axis (Nelson *et al.*, *Advances in Protein Chemistry*, vol. 73, pp. 235-282, 2006, and Guijarro *et al.*, *PNAS*, 95, no. 8, pp. 4224-4228, 1998). K^+ and K^- , separately, also showed two peaks based on the 1D WAXS radial intensity distribution at the same d -spacing of $d = 0.96$ nm (intersheet spacing) and $d = 0.46$ nm (interstrand spacing). However, due to the isotropic nature of the K^+ and K^- samples, they do not assemble into amyloid structures unlike the CK and KK fibers, further validated by the results of Congo red staining as explained above. Hence, the two peaks recorded in K^+ and K^- samples only proved that they consisted of β -sheet structures. This was in good agreement with Pena-Francesch *et al.* (*ACS Biomaterials Science & Engineering*, vol. 4, no. 3, pp. 884-891, 2018) who reported similar isotropic WAXS diffraction pattern and d -spacing for squid ring teeth-inspired proteins which presented β -sheet structures. Additionally, Cho *et al.* (*Nature Communications*, vol. 6, no. 1, pp. 1-7, 2015) and Boni *et al.* (*ACS Applied Materials & Interfaces*, vol. 10, no. 47, pp. 40460-40473, 2018) also reported similar d -spacing values for silk proteins and hagfish films, represent β -sheet structures without amyloids. Furthermore, the WAXS results for CK, KK, K^+ and K^- were consistent with the ATR-FTIR findings where the majority proportion of secondary structures were concluded to be β -sheets based on deconvolution of the Amide I peak (FIG. 2(c)).

Example 4: Morphology

Field Emission Scanning Electron Microscopy (FESEM)

FESEM (JEOL JSM-6340F) was used to observe the morphology and cross-section of the fibers. An emission current and accelerating voltage of 12 μ A and 5 kV were used respectively. Prior to imaging, all fibers were gold sputtered for 10 seconds at 20 mA. All images were taken in Lower Secondary Electron (LEI) mode.

Results and Discussion

Based on the FESEM morphological images, both CK (FIG. 4(a)) and KK (FIG. 4(b)) fibers exhibited the characteristic nervation pattern common in fibers formed by IPC, which reflected the highly aligned nature the fibers. This nervation pattern was a result of fusing 10 fibrils together and were morphologically similar to other reported IPC fiber compositions (Do *et al.*, *Advanced Functional Materials*, vol. 27, no. 42, pp. 1-10, 2017 and Tai *et al.*, *Biomaterials*, vol. 31, no. 23, pp. 5927-5935, 2010). Based on the cross-section of CK (FIG. 4(c)) and KK (FIG. 4(d)) fibers, the 10 individual fibrils that made up the fiber were well integrated together and could not be differentiated visually. This was due to effective inter-fibril fusion as they were spooled onto one another in the wet state and fused together when dried. Also, the cross-section of the fibers were homogeneous, which indicated that there was no phase separation of the oppositely charged polyelectrolytes. These observations were similar to the morphology of reported chitosan-heparin IPC bundled fibers of various numbers of fused fibrils, which could not be discerned due to the effective fusion of fibrils (Do *et al.*, *Advanced Functional Materials*, vol. 27, no. 42, pp. 1-10, 2017). The IPC fibers were flexible and versatile enough to be weaved into a mesh (FIG. 4(e)), knotted (FIG. 4(f)) and braided (FIG. 15) manually using tweezers. The various morphologies produced showed the potential for the CK and KK fibers to be used in various biomedical applications such as sutures and resorbable meshes.

Example 5: Tensile Properties of the CK and KK fibers

Tensile Test

The tensile properties of the CK and KK fibers consisting of 10 fibrils were measured with a mechanical tester (MTS Criterion Model C42). Individual dried fibers were glued onto a cardboard frame with gauge length of 20 mm. The cardboard frame was mounted on 200 N pneumatic grips and a 10 mm/min drawing speed was applied by a 50 N load cell. A light microscope (IX53, Olympus) was used to measure the dried fiber diameter at 10 random points. Stress was calculated by using the average fiber cross-sectional area based on the mean diameter. 10 fibers for each fiber type (KK or CK) were tested. Only measurements obtained from fibers where failure occurred at the centre of the gauge length were counted.

The Young's modulus, UTS, Strain-at-break, and Max Load were calculated using the mechanical tester (MTS Criterion Model C42) for each fiber. In brief, the Young's modulus was calculated from the gradient of two points within the elastic region of the stress-strain curve; UTS was calculated from the maximum stress within the plastic region of the stress-strain curve; strain-at-break was calculated from the maximum strain prior to breakage; and Max Load was calculated from the maximum load within the plastic region of the stress-strain curve.

Results and Discussion

Individual CK and KK fibers were glued onto a cardboard frame and tested for its tensile properties using the mechanical tester. Only readings where fibers broke in the middle of the gauge length were recorded. From the representative stress-strain curves (FIG. 5(a)) CK fibers recorded a mean Young's modulus of 2.0 ± 0.2 GPa, which was significantly higher compared to 0.9 ± 0.2 GPa recorded for KK fibers due to the stronger electrostatic interaction between C+ and K- (FIG. 5(b)). The Ultimate Tensile Strengths (UTS) of CK and KK fibers (FIG. 5(c)) were comparable, at 32.1 ± 5.5 MPa and 28.6 ± 6.5 MPa, respectively. Both fibers had large proportions of β -sheet secondary structures (FIG. 2(c)) which were stabilized by hydrogen bonds, resulting in significant strength of the fibers. Non-absorbable sutures such as polypropylene and nylon have UTS values of 493.1 and 656.5 MPa while absorbable sutures such as PLGA and chromic gut have UTS values of 1377.0 and 410.0 MPa.

KK fibers had a significantly larger mean strain-at-break value ($96.6 \pm 17.2\%$) compared to CK fibers ($16.1 \pm 6.4\%$) (FIG. 5(d)) due to the strain-stiffening effect. This occurred as the long molecular chains of K+ and K- behaved similarly to classical linear polymer chains that untangle and straighten out to become increasingly stiff with increasing strain applied. These mechanical property trends were comparable across two other keratin extraction batches, demonstrating reproducibility (FIG. 16). Other chitosan-based IPC fibers behaved in a similar fashion. For example, Chitosan-heparin IPC fibers were reported to have a high UTS of 220 MPa, a much lower strain-at-break value of 11.5% (Do *et al.*, *Advanced Functional Materials*, vol. 27, no. 42, pp. 1-10, 2017). Likewise, chitosan and TEMPO-oxidized cellulose nanofibrils IPC fibers recorded a high Young's modulus of 15 GPa, a UTS of 200 MPa but a strain-at-break value of 9.2% (Toivonen *et al.*, *Biomacromolecules*, vol. 18, no. 4, pp. 1293-1301, 2017). Expectedly, the crystalline nature of polysaccharides renders rigidity and brittleness to chitosan in the CK fibers. In comparison, complementary proteinous materials such as K+ and K- impart greater flexibility and stretchability to the fibers. Overall, in terms of mechanical properties, the CK and KK fibers had lower UTS but similar Young's moduli and strain-at-break values compared to commercial sutures. Nonetheless, our functional study demonstrated that these mechanical properties are adequate for the suture application. Where needed, fiber diameters, and hence the load capacity of the fibers, can be tuned by adjusting fiber drawing parameters.

The mechanical properties of four different configurations of the CK and KK fibers (single fibril fiber, 10-fibril fiber, 30-fibril fiber and 50-fibril fiber) are shown in Table 1 below. The data is presented as means $\pm$ standard deviation ($n=10$ for single and 10-fibril fibers, $n=5$ for 30- and 50-fibril fibers).

Table 1: The mechanical properties of four different configurations of the CK and KK fibers

Sample	Max Load (N)	Young's Modulus (GPa)	UTS (MPa)	Strain at Break (%)
CK single fibril fiber	0.07 ± 0.04	2.36 ± 0.76	28.51 ± 12.47	4.43 ± 1.44
CK 10-fibril fibers	0.46 ± 0.15	2.05 ± 0.23	32.12 ± 5.53	16.08 ± 6.44
CK 30-fibril fibers	3.12 ± 0.98	0.61 ± 0.19	38.61 ± 7.93	20.48 ± 3.09
CK 50-fibril fibers	5.34 ± 0.47	0.78 ± 0.33	41.39 ± 6.28	35.67 ± 11.75
KK single-fibril fiber	0.06 ± 0.02	2.05 ± 0.26	39.06 ± 12.74	39.09 ± 23.70
KK 10-fibril fibers	0.33 ± 0.08	0.88 ± 0.19	28.56 ± 6.51	96.63 ± 17.24
KK 30-fibril fibers	0.98 ± 0.38	0.49 ± 0.17	13.17 ± 4.56	92.20 ± 52.76
KK 50-fibril fibers	3.54 ± 0.62	0.50 ± 0.09	21.40 ± 6.61	31.47 ± 8.54

The diameter of the fibers used in the tensile strength experiments were measured. The results are presented in Table 2 below.

Table 2: Dried fiber diameters of the fibers used in the tensile strength experiments. The diameter was recorded for each fiber at 10 different points along the length of a fibre.

Sample No.	CK Fiber Diameter (μm)	KK Fiber Diameter (μm)
1	155.758	155.759
	156.364	152.122
	159.399	149.091
	144.546	136.365
	142.728	152.121
	141.819	157.577
	150.91	153.333
	151.516	156.364
	153.333	153.333
	150.308	151.516
	Average: 150.7	Average: 151.8
2	170.455	149.698
	165.465	150.909
	165.932	153.335
	145.456	149.698
	145.456	152.122
	149.697	157.576
	153.636	156.971
	145.909	144.092
	147.728	143.637
	152.727	146.364
	Average: 154.2	Average: 150.4
3	146.668	164.85
	145.456	162.425

	155.153 133.941 136.369 135.758 145.456 144.848 144.85 146.061 Average: 143.5	160 161.212 166.062 168.486 169.092 166.668 169.092 170.304 Average: 165.8
4	153.335 153.333 150.303 157.273 154.546 155.91 149.545 131.831 130 129.546 Average: 146.6	151.516 155.156 156.364 157.577 158.793 151.516 151.819 154.545 149.546 153.637 Average: 154.0
5	148.637 149.546 154.092 146.819 154.546 150 148.637 147.728 149.091 147.273 Average: 149.6	139.546 139.546 143.183 199.394 202.425 192.728 148.183 152.273 146.381 146.819 Average: 161.0
6	179.546 180.91 179.545 180 184.242 177.577 178.789 175.152 173.94 169.092 Average: 177.9	156.364 155.455 155.455 154.546 152.121 154.547 158.183 137.576 140.606 138.789 Average: 150.4
7	171.364 170.001 165.001 136.364 142.727 150.455 157.728 157.273 153.182 158.637 Average: 156.3	165.909 164.548 164.546 169.697 155.758 166.062 149.697 147.274 147.879 149.698 Average: 158.1

8	143.032	172.125
	140	169.697
	133.941	170.909
	166.364	156.374
	160.91	160.919
	158.182	158.198
	152.273	154.556
	151.818	158.647
	153.182	163.192
	157.728	156.819
	Average: 151.7	Average: 162.1
9	158.182	182.424
	158.182	172.732
	165.001	167.274
	153.182	164.85
	152.273	158.788
	156.364	160.606
	157.727	165.456
	165	167.88
	164.546	172.766
	165.001	178.265
	Average: 159.5	Average: 169.1
10	158.182	158.788
	158.192	158.182
	158.845	162.424
	153.941	138.183
	154.545	135.152
	150.92	134.545
	149.697	156.364
	146.061	151.818
	149.698	156.364
	149.698	160.455
	Average: 153.0	Average: 151.2

All of the fibers tested were found to have average diameters within the range of 140 μm to 180 μm , when in dried form.

Example 6: Thermal Properties

Differential Scanning Calorimetry (DSC)

Thermal properties for the three polyelectrolyte solutions (K+, K- and C+) and fibers (CK and KK) were measured with DSC (DSC Q10, TA Instruments). The polyelectrolyte solutions were freeze-dried and fibers were air dried prior to testing. An aluminium pan was used to encase each sample and an empty aluminium pan was used as reference. Each sample was equilibrated to 25 °C and subsequently subjected to a 10 °C/min heating rate up till 400 °C.

Thermogravimetric Analysis (TGA)

Decomposition temperature of the three polyelectrolyte solutions (K+, K- and C+) and fibers (CK and KK) were determined with TGA (TGA 2950, TA Instruments). The polyelectrolyte solutions were freeze-dried and fibers were air dried prior to testing. An alumina crucible was used to encase each sample. This was equilibrated to 25 °C and subsequently subjected to a 10 °C/min heating rate up till 900 °C.

Results and Discussion

Thermal stability of the polyelectrolyte solutions as well as the corresponding CK and KK fibers were evaluated with DSC (FIG. 6(a)). Based on the DSC thermographs, the temperatures of the endothermic and exothermic peaks were tabulated (Table 3 and 4).

Table 3: The measured temperature values of the endothermic and exothermic peaks for the various samples as obtained from DSC, n = 3, data is presented as means $\pm$ standard deviation.

Fiber type	Endothermic [Water Evaporation] (°C)	Endothermic [α -helix Denaturation] (°C)	Exothermic [Decomposition of Amine] (°C)	Endothermic [β -sheet Denaturation] (°C)
CK	87.5 $\pm$ 3.1	228.6 $\pm$ 11.4	284.6 $\pm$ 6.8	319.2 $\pm$ 9.2
KK	111.2 $\pm$ 9.9	209.7 $\pm$ 5.0	-	318.8 $\pm$ 3.3
C+	88.0 $\pm$ 2.4	-	291.6 $\pm$ 0.0	-
K-	88.6 $\pm$ 3.1	236.0 $\pm$ 2.8	-	310.4 $\pm$ 6.2
K+	120.7 $\pm$ 4.5	212.7 $\pm$ 4.7	-	304.1 $\pm$ 8.9

Table 4: The measured weight loss and decomposition temperature for the various samples as obtained from TGA, n = 3, data is presented as means $\pm$ standard deviation.

Fiber type	First Stage of Weight Loss (%)	Second Stage of Weight Loss (%)	Decomposition Temperature (°C)
CK	11.3 $\pm$ 1.3	64.9 $\pm$ 0.7	338.7 $\pm$ 1.7
KK	9.9 $\pm$ 0.4	68.5 $\pm$ 1.9	345.0 $\pm$ 2.2
C+	15.6 $\pm$ 1.0	48.9 $\pm$ 1.8	307.1 $\pm$ 5.1
K-	8.7 $\pm$ 0.7	71.3 $\pm$ 0.9	351.8 $\pm$ 6.0
K+	3.8 $\pm$ 0.1	39.5 $\pm$ 0.8	318.4 $\pm$ 22.5 and 363.4 $\pm$ 9.4

All samples exhibited an endothermic peak around 87.5 °C to 120.7 °C due to evaporation of water which was indicated by region 1 (FIG. 6(a)). For C+, an exothermic peak occurred at 291.6 °C due to the decomposition of glucosamine units of chitosan indicated by region 3. This exothermic peak was also reflected in the CK fiber at 284.6 °C which showed the incorporation of C+ in the fiber. An endothermic peak was observed between 209.7 °C and 236.0 °C for K+,

K-, CK and KK, indicating α -helix denaturation in the keratins which was shown by region 2. Another endothermic peak was observed between 304.1 °C and 319.2 °C for K+, K-, CK and KK which indicated β -sheet denaturation which was shown by region 4 (see Table 3).

The thermal decomposition of polyelectrolyte solutions (K+, K- and C+) and fibers (CK and KK) were measured with TGA (FIG. 6(b)). Two weight loss regions were identified for all samples (see Table 4). The first stage of weight loss, indicated by region 1 (FIG. 6(b)), occurred from 25 °C to 100 °C due to water evaporation and results in a weight loss of 3.8% to 15.6% of the samples. For the case of C+, an additional decomposition of acetic acid occurred at 150.7 ± 2.4 °C which accounted for $12.7 \pm 0.8\%$ of weight loss. For the case of K+, an additional decomposition of citric acid occurred at 185.4 ± 6.7 °C which resulted in a $45.2 \pm 2.1\%$ weight loss.

The second stage of weight loss indicated by region 2 happened from 200 °C to 400 °C due to cleavage of the polymer backbone resulting from polymer degradation. This resulted in a 39.5% to 71.3% weight loss in the samples. The thermal decomposition of C+ occurred at 307.1 °C due to dehydration of the cyclic rings in the glucosamine repeat units. The amount of weight loss was around 48.9%, similar to reported values of around 51% (Bell *et al.*, *Advanced Materials Interfaces*, vol. 7, no. 23, pp. 1-9, 2020). K- decomposed at 351.8 °C while K+ decomposed between 318.4 °C to 363.4 °C. The decomposition temperature for CK fibers occurred at 338.7 °C, which was between the decomposition temperature of its components C+ and K-. The decomposition of KK fibers occurred at 345.0 °C and this was between the decomposition temperatures of its components K+ and K-. Above 600 °C, stagnant weight loss indicated the burning of C+, K+ and K- polyelectrolyte solutions and the CK and KK fibers. Based on the DSC and TGA results, CK and KK fibers are thermally stable to be used in biomedical applications.

Example 7: *In vivo* wound closure performance of CK and KK fibers

Wound Closure Experiment

The current experiment was carried out to evaluate the suitability of the CK and KK fibers as sutures. CK and KK fibers of 10 cm (USP size 5-0, 150-199 μ m) in length were produced by spooling the fibers onto a Teflon rod of diameter 3.5 cm. The CK and KK fibers were evaluated for its ability to close a wound. The control suture was a commercially available polyamide suture (4-0 Dafilon®; B BRAUN; 150-199 μ m). A total of 15 C57BL/6J female mice were anaesthetized with isoflurane inhalation. 10 mice (5 for CK, 5 for KK) were euthanized on Day 3 and 5 mice (3 for CK, 2 for KK) were euthanized on Day 7. A 10 mm incision was made on

the dorsal skin of each mouse after shaving. Each incision was closed by 5 surgical knots with alternating suture material consisting of the CK or KK fibers and the control suture. Tegaderm was used to protect the wound sites. On the respective time points of Day 3 and Day 7, the mice were euthanized. The dorsal skin was harvested, fixed in 4% paraformaldehyde and embedded in paraffin. A microtome was used to slice the tissue into 5 μ m thick sections. These tissue sections were processed routinely for Haematoxylin and Eosin (H&E) staining and Masson's Trichrome staining.

Results and Discussion

CK and KK fibers were flexible and able to withstand knotting, indicating suitability as a surgical suture (FIG. 7). In a mouse skin incisional wound model, both CK and KK fibers were able to keep the wound close which showed that they had sufficient mechanical strength. No acute inflammation was observed at the incision sites at Day 3 (FIG. 17: cross sectional view along wound incision) and Day 7 (FIG. 18: cross sectional view along wound incision) post-procedure.

Based on H&E staining it was observed that the polyamide sutures as well as the CK and KK fibers were clearly present within the scab (above the epidermis) and within the dermis or panniculus carnosus layer at Day 3 post procedure (FIG. 17). Based on Masson's trichrome staining, the CK fibers were more clearly encased in a dense dark purple layer consisting of inflammatory cells around the fiber compared to both KK fibers and the polyamide suture (FIG. 17), suggesting a more pronounced inflammation phase at the wound site sutured using the CK fiber. Fibrotic encapsulation was not present around all samples.

At Day 7, there was successful wound closure across all samples, as seen by the complete healing of the epidermis and dermis (FIG. 7). Inflammatory cells such as neutrophils indicated by the grey arrows and fibroblasts (spindle morphology, large euchromatic nuclei and the basophilic cytoplasm) indicated by the black arrows were present at the wound closure site for all three samples (FIG. 7). Masson's trichrome staining showed that the wounds have all progressed into the remodelling phase (FIG. 18), with collagen deposition shown at the incision site by the purple-blue staining. Overall, unremarkable differences were observed between the 3 sample groups, suggesting the feasibility of using both KK and CK fibers as sutures, comparable to polyamide. At this point, the scabs have fallen off and the sutures were no longer visible (FIG. 18). Instead of degradation, it was concluded that the materials were pulled off by the mice as it was also observed that the covering Tegaderm sheets were also removed. This deduction was corroborated against the biodegradation results described in the following Example 8.

Example 8: *In vivo* biodegradation of CK and KK fibers***Biodegradation Experiment***

The current experiment was carried out to evaluate the biodegradation profile of the CK and KK fibers. CK and KK fibers (USP size 5-0) of 1 cm length were prepared. The control was commercially available polyglyconate (4-0 Monosyn®; B BRAUN; 150-199 µm). A total of 34 C57BL/6J female mice were anaesthetized with isoflurane inhalation. 12 mice (6 for CK, 6 for KK) were euthanized on Day 7, 12 mice (6 for CK, 6 for KK) were euthanized on Day 14 and 10 mice (5 for CK, 5 for KK) were euthanized on Day 21. One 10 mm incision was made on the dorsal skin of each mouse after shaving. One IPC fiber was placed in the subcutaneous pocket of each mice and the control suture was used to close the incision. After each time point (Day 7, 14 and 21), the mice were euthanized. The dorsal skin was harvested, fixed in 4% paraformaldehyde and embedded in paraffin. A microtome was used to slice the tissue into 5 µm thick sections. These tissue sections were processed routinely for Haematoxylin and Eosin (H&E) staining and Masson's Trichrome staining.

Results and Experiment

CK and KK fibers were subcutaneously implanted under the dorsal skin of mice. Both fibers remained intact and did not evoke excessive host tissue response post implantation over 21 days. Based on H&E and Masson's Trichrome staining, CK fibers were observed to elicit thick fibrous capsule formation after 7 and 14 days (FIG. 19 and FIG. 20) post implantation compared to KK fibers which induced relatively benign tissue response that was comparable to that of the polyamide suture. A significant number of leukocytes indicated by the grey arrows as well as fibroblasts indicated by the black arrows were recruited around the periphery of CK fibers over the 21 day duration (FIG. 8). Immune cell recruitment around KK fibers and the polyamide sutures was again comparable and relatively mild. While no cellular infiltration was observed into both CK and KK fibers, a dense cell layer containing foreign body giant cells (FBGC) was observed around the CK fibers, among fragments of the fiber material which was stained bright pink. This suggested active degradation of the CK fiber, which resulted in the appearance of jagged boundaries of the fiber across all 3 time points. Notably, the thickness of the fibrous capsule layers around CK fibers decreased by Day 21. Similar degradation at the periphery of the KK fibers was also observed albeit the presence of FBGC was more subdued, and fragmentation of the fiber itself was less pronounced. Interestingly, the extent of immune cell recruitment and fibrous capsule encapsulation around the KK fiber samples were as muted as that observed around the non-absorbable commercial polyamide suture across the implantation duration.

Previous work had demonstrated *in vivo* biocompatibility of different hair keratin templates. Keratin hydrogels that were subcutaneously implanted into the dorsal skin of wild type mice showed good integration with the surrounding host tissue and cellular infiltration into the hydrogel with remodelling (Wang *et al.*, *ACS Applied Materials & Interfaces*, vol. 7, no. 9, pp. 5187-5198, 2015). These gels persisted subcutaneously in rats, presenting fragmented pieces with mild fibrotic encapsulation after 90 days, similar to the level of encapsulation observed around the KK fibers in the current study. Separately, keratin-alginate composite sponges were capable of supporting cellular infiltration and neovascularization over 4 weeks subcutaneously in mice (Hartrianti *et al.*, *Journal of Tissue Engineering and Regenerative Medicine*, vol. 11, no. 9, pp. 2590-2602, 2017). Keratin scaffolds subcutaneously implanted into mice showed no swelling or irritation and there was cellular infiltration and new blood vessel formation. A thin fibrous capsule layer was also present around the scaffold which was stained by Masson's trichrome (Guzman *et al.*, *Biomaterials*, vol. 32, no. 32, pp. 8205-8217, 2011). Keratin hydrogels implanted into a diabetic rat showed accelerated wound healing as re-epithelialization was observed in H&E stained tissues and collagen formation was observed from Masson's trichrome staining (Chen *et al.*, *Acta Biomaterialia*, vol. 125, pp. 208-218, 2021).

Taking the preliminary *in vivo* results in totality, both CK and KK fibers could hold skin incisional wounds closed, and do not elicit severe host tissue response, which suggested potential to function effectively as suture materials. KK fibers appeared to perform better than CK fibers due to the lower levels of inflammation that was comparable with established polyamide sutures. Furthermore, KK fibers degraded slower than CK fibers as seen from their intact form up to 3 weeks post implantation while CK fibers fragmented within the fibrous capsule layer.

Example 9: Optimisation of polyelectrolyte concentration and volume to volume ratio of polycation (C+ or K+) to polyanion (K-)

The preparation of the CK and KK fibers using IPC was optimised. As shown in FIG. 10 and 11, the concentrations for the polyelectrolyte solutions used in the preparation of the fibers influenced the length of the fiber that could be drawn from the IPC interface.

Specifically, various concentrations of C+ and K- were used at 1:1 v/v ratio (with respect to each other) to produce CK fibers and the corresponding length of CK fiber produced was measured. FIG. 10(b) includes the various concentrations of K+ and K- used at 1:1 v/v ratio (with respect to each other) to produce KK fibers and the corresponding length of KK fiber produced. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * p < 0.05; data is presented as means ± standard deviation (N = 3, n = 3).

In addition, various v/v ratio of 25 mg/ml C+ and 9 mg/ml K- were used to produce CK fibers and the corresponding length of CK fiber produced was measured. FIG. 11(b) includes the various v/v ratio of 5 mg/ml K+ and 9 mg/ml K- used to produce KK fibers and the corresponding length of KK fiber produced. Means comparison was done with One-way ANOVA and Tukey's post hoc test; * $p < 0.0$; data is presented as means $\pm$ standard deviation ($N = 3$, $n = 3$). A polycation (K+/C+) to polyanion (K-) v/v ratio of 1:1 was found to be optimal for maximum fibre length.

Example 10: Additional experimental data

Oppositely charged polyelectrolyte solutions were brought together in various weight ratios as listed in Table 5. K+ has a concentration range of between 4 to 6 mg/ml. K- has a concentration range of 9 to 11 mg/ml. C+ has a concentration range of 12.5 to 25 mg/ml.

Table 5: Weight ratios of polycations and polyanions, and the number of rotations around a Teflon rod to form various CK and KK fibers.

Polycation	Polyanion	Weight ratio of Polycation to Polyanion	Number of Rotations	IPC Combination
C+	K-	0.5	10	CK1
C+	K-	2.7	10	CK2
C+	K-	13.8	10	CK3
K+	K-	0.1	10	KK1
K+	K-	0.5	10	KK2
K+	K-	2.7	10	KK3

The diameter for various CK and KK fiber combinations for a spooled fiber consisting of 10 rotations around the dropper is listed in Table 6. For the same number of rotations, it was observed that CK fibers had a smaller diameter compared to KK fibers. This shows that the long and continuous CK fibers that are produced are much thinner compared to KK fibers which are short and fat. Single CK fibers had a diameter of $30.56 \pm 2.06 \mu\text{m}$ ($n=100$) while single KK fibers had a diameter of $45.98 \pm 4.35 \mu\text{m}$ ($n=100$).

Table 6: Dried fiber diameter of various IPC combinations consisting of 10 rotations.

IPC Combination	Fiber Diameter (μm)
CK1	118.00 ± 5.94
CK2	110.50 ± 11.62
CK3	90.50 ± 15.57
KK1	147.91 ± 18.90
KK2	148.09 ± 24.16

KK3	171.86±9.05
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The Young's modulus (FIG. 21(a)) and UTS (FIG. 21(b)) for all CK fibers were much higher than KK fibers. This is unexpected as CK fibers are also thinner compared to KK fibers. It would have been expected that the thicker fibers should have greater tensile strength. However, a possible explanation for the CK fibers having better Young's modulus and UTS could be due to the larger zeta potential difference (FIG. 1(b)) and high viscosity of C+ (FIG. 1(c)). The larger zeta potential difference between C+ and K- would suggest that the fibers are formed with greater electrostatic interaction and are more stable. In addition, the high viscosity of C+ also provides another strengthening mechanism to the CK fiber.

The strain at break (FIG. 21(c)) for all KK fibers were much higher than CK fibers. A possible explanation could be due to the fact that KK fibers are not made from a continuous spool of fiber unlike CK fibers. All CK fibers could be spooled continuously until 10 rotations. KK fibers would often break after two to four rotations, hence additional spools would need to be added on to achieve 10 rotations. This also resulted in the fiber diameters of KK fibers which were thicker than CK fibers. The addition of more spools in KK fibers could have caused its increase in flexibility and allowed the fiber to be stretched longer before all fibers broke. This is seen in FIG. 5(a) which showed the KK fiber under strain for a longer time compared to CK fiber. This suggests that the individual strands within the spooled KK fiber could stretch and break a few at a time, hence prolonging the time before a complete fracture of the fiber occurs.

The tensile properties of existing suture materials are shown in Table 7. The Young's Modulus of the CK and KK fibers is comparable to existing suture materials such as polypropylene, nylon, polyglyconate and chromic gut. The strain at break is also similar to other suture materials. However, the UTS of IPC fibers are lower than existing suture materials.

Table 7: Mechanical properties of sutures (Naleway *et al.*, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 103, no. 4, pp. 735-742, 2015).

Material	Young's Modulus (MPa)	Ultimate Tensile Strength (MPa)	Strain at Break (%)
Non-absorbable Sutures			
Polypropylene	745.6	493.1	94.8
Polybutester	945.3	595.3	98.4
Nylon	1163.9	656.5	99.4
Silk	8701.0	1136.3	26.6
Absorbable Sutures			
Polyglyconate	393.3	726.3	93.6
Polydioxane	397.8	557.5	144.2
Chromic Gut	750.1	410.0	52.0

Polyglactin 910	4464.0	1377.0	52.8
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Conclusion

Keratins originating from human hair have been processed into various forms for biomedical applications such as scaffolds for tissue engineering. In the present disclosure, the technique of IPC was adopted to produce two novel composite fibers based on combinations of hair keratin and chitosan. These fibers were thoroughly characterized to understand their physico-chemical and mechanical properties. The chitosan-keratin (CK) fibers registered tensile strength of 32 MPa, Young’s modulus of 2 GPa and strain-at-break of about 16% while the keratin-keratin (KK) fibers exhibited tensile strength of 29 MPa, Young’s modulus of 0.9 GPa and strain-at-break of about 100%. The predominant secondary structure found in both fibers was β -sheets which resulted in their good mechanical strength. In fact, the keratins in these fibers were found to assemble into amyloid-like structures, proven by the green birefringence of Congo red stained fibers under cross-polarized light as well as the cross- β diffraction pattern observed in WAXS. The ability of the CK and KK fibers to be braided, weaved and knotted indicated their malleability and versatility to be used for various biomedical applications. In particular, the feasibility of using these fibers as medical sutures was demonstrated *in vivo* herein. This was shown by the successful closure of incisional wounds on the back of wild type mice without eliciting significant host-tissue response. Both fibers degraded slowly over 3 weeks post implantation with the CK fibers degrading faster than KK fibers. Furthermore, CK fibers were encased in a thicker fibrous capsule layer compared to KK fibers. Most significantly, the extent of immune cell recruitment and fibrous capsule encapsulation evoked by the KK fiber were mild and comparable to non-absorbable polyamide sutures. The different properties of CK and KK fibers show the possibility to tune the mechanical properties and degradation rate of the fibers based on the intended application. Overall, this work presented novel keratin based micro-scale composite fibers produced using the IPC technique. The fibers were evaluated to have the potential for application as medical sutures.

CLAIMS

1. A fiber comprising two or more fibrils,
the two or more fibrils each comprising an anionic α -keratin intermediate filament and a cationic polymer,
the cationic polymer selected from the group consisting of a cationic polysaccharide and a cationic α -keratin intermediate filament.
2. The fiber of claim 1, wherein the fiber comprises at least 5 fibrils, for example, about 10 fibrils.
3. The fiber of claim 1 or 2, wherein the cationic polymer is a cationic α -keratin intermediate filament.
4. The fiber of claim 1 or 2, wherein the cationic polymer is a cationic polysaccharide.
5. The fiber of claim 4, wherein the cationic polysaccharide is cationic chitosan.
6. The fiber of any one of the preceding claims, wherein the anionic α -keratin intermediate filament, and the cationic α -keratin intermediate filament when present, are each obtained from a mammalian source, for example a human source.
7. The fiber of any one of the preceding claims, wherein the anionic α -keratin intermediate filament, and the cationic α -keratin intermediate filament when present, are each derived from mammalian hair, for example human hair.
8. The fiber of any one of the preceding claims, wherein the anionic α -keratin intermediate filament, and the cationic α -keratin intermediate filament when present, are each substantially free from keratin associated proteins.
9. The fiber of any one of the preceding claims, wherein the anionic α -keratin intermediate filament, and the cationic α -keratin intermediate filament when present, are each substantially free from lipids.

10. The fiber of any one of the preceding claims, wherein the fiber is formed from about 10 fibrils and has a cross-sectional diameter of from about 70 μm to about 200 μm , for example, from about 150 μm to about 200 μm .
11. The fiber of any one of the preceding claims, wherein the fiber has a homogenous cross-section.
12. The fiber of any one of the preceding claims, wherein each fibril is formed by interfacial polyelectrolyte complexation.
13. A suture comprising one or more fibers according to any one of claims 1 to 12.
14. A mesh comprising one or more fibers according to any one of claims 1 to 12.
15. A method of preparing the fiber according to any one of claims 1 to 12, said method comprising:
 - (a) providing an aqueous solution of the anionic α -keratin intermediate filament and an aqueous solution of the cationic polymer;
 - (b) contacting the aqueous solution of the anionic α -keratin intermediate filament and the aqueous solution of the cationic polymer under conditions conducive to form an interface; and
 - (c) pulling a fibril formed from the anionic α -keratin intermediate filament and the cationic polymer from the interface;
 - (d) forming the fiber according to any one of claims 1 to 12 by combining two or more portions of the fibril from step (c) or combining two or more fibrils formed by repeating steps (a) to (c).
16. The method according to claim 15, wherein step (a) further comprises providing a sample of mammalian hair, followed by extracting lipids from the sample using an organic solvent and extracting keratin associated proteins from the sample using a denaturing agent, thereby providing a sample of α -keratin intermediate filaments.
17. The method according to claim 16, wherein step (a) further comprises reducing the sample of α -keratin intermediate filaments with a reducing agent.

18. The method according to any one of claims 16 or 17, wherein step (a) further comprises providing the sample of α -keratin intermediate filaments in an aqueous solution, followed by exchanging the aqueous solution with deionised water, thereby providing the aqueous solution of the anionic α -keratin intermediate filament.
19. The method according to any one of claims 15 to 18, wherein the aqueous solution of the anionic α -keratin intermediate filament has an anionic α -keratin intermediate filament concentration of from about 4 mg/ml to about 15 mg/ml, for example about 9 mg/ml.
20. The method according to any one of claims 15 to 19, wherein the cationic polymer is a cationic α -keratin intermediate filament.
21. The method according to claim 20 when dependent on claim 16, wherein step (a) further comprises providing the sample of α -keratin intermediate filaments in an aqueous solution, followed by reducing the pH of the aqueous solution to a pH of below about 4.5, thereby providing an aqueous solution of the cationic α -keratin intermediate filament.
22. The method according to claim 20 or 21, wherein the aqueous solution of the cationic polymer has a cationic α -keratin intermediate filament concentration of from about 1 mg/ml to about 12 mg/ml, for example about 5 mg/ml.
23. The method according to any one of claims 15 to 19, wherein the cationic polymer is a cationic polysaccharide, for example cationic chitosan.
24. The method according to claim 23, wherein step (a) further comprises providing a sample of a polysaccharide, followed by dissolving the polysaccharide in an aqueous acid solution, thereby providing an aqueous solution of the cationic polysaccharide.
25. The method according to claim 24, wherein the aqueous solution of the cationic polysaccharide has a cationic polysaccharide concentration of from about 10 mg/ml to about 30 mg/ml, for example, about 25 mg/ml.

26. The method according to any one of claims 15 to 25, wherein step (d) comprises spooling the fibril onto itself, thereby combining two or more portions of the fibril to form the fiber.