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Wet-spinnability and crosslinked fibre properties of two collagen polypeptides with varied molecular weight

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ABSTRACT

The formation of naturally derived materials with wet stable fibrous architectures is paramount in order to mimic the features of tissues at the molecular and microscopic scale. Here, we investigated the formation of wet-spun fibres based on collagen-derived polypeptides with comparable chemical composition and varied molecular weight. Gelatin and hydrolysed fish collagen (HFC) were selected as widely available linear amino-acidic chains of high and low molecular weight, respectively, and functionalised in the wet-spun fibre state in order to preserve the material geometry in physiological conditions. Wet-spun fibre diameter and morphology were dramatically affected depending on the polypeptide molecular weight, wet-spinning solvent (i.e. 2,2,2-trifluoroethanol and dimethyl sulfoxide) and coagulating medium (i.e. acetone and ethanol), resulting in either bulky or porous internal geometry. Dry-state tensile moduli were significantly enhanced in gelatin and HFC samples following covalent crosslinking with activated 1,3-phenylenediacetic acid (Ph) (E: 726 ± 43–844 ± 85 MPa), compared to samples crosslinked via intramolecular carbodiimide-mediated condensation reaction (E: 588 ± 38 MPa). Resulting fibres displayed a dry diameter in the range of 238 ± 18–355 ± 28 μm and proved to be mechanically stable (E: 230 kPa) following equilibration with PBS, whilst a nearly complete degradation was observed after 5-day incubation in physiological conditions.

1. Introduction

Polypeptides are essential components in various biological materials and play a major role in structural stabilisation, energy conversion and tissue remodelling [1]. Polypeptides derived from the organic matrix of tissues, such as gelatin or hydrolysed collagen are widely available, biodegradable and contain cell-binding sequences, making them appealing building blocks for the design of healthcare products found in applications such as chronic wound care, guided bone regeneration (GBR), and stratified medical devices. The integration of such building blocks into defined fibrous architecture is therefore of high technological and scientific interest in order to mimic tissue organisation at different length scales and accomplish material systems with unexpected properties and functionalities.

Polypeptide-based fibrous architectures can typically be prepared via solution-based spinning techniques, whereby two main strategies can be pursued: (i) direct formation of nano-/submicron webs by electrospinning [2]; (ii) fibre spinning, via methods such as dry- [3], gel- [4], or wet-spinning [5–7], potentially combined with post-drawing [8] or electrophoretic compaction [9], whereby fibres are obtained and used to make customised fabrics. Both strategies have been successfully applied to gelatin as collagen-derived polypeptide, resulting in spirally electrospun webs [10] as well as bulky wet-spun fibres [5] with porous morphology and aligned polypeptide chains. Here, continuous gelatin filaments were successfully formed via a ternary system of water, isopropanol and plasticiser, whereby superior and tunable dry-state tensile moduli (up to nearly 4 GPa) were obtained depending on the wet-spinning mixture formulation and plasticiser content.

Electrospinning has received a great deal of attention because it is simple to operate and inexpensive, and can process a range of polymers [11]. It also enables the formation of cell-laden webs via co-spinning of synthetic polymers with cell suspensions [12], an approach currently refereed as "cell electrospinning" [13]. Despite largely applicable to synthetic polymers, however,

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electrospinning of polypeptides offers restricted possibilities with regard to architecture customisation, three-dimensional geometry and morphology preservation in physiological environments [14]. Other than electrospinning, classical fibre spinning methods, such as dry- or wet-spinning, can lead to the formation of defined fibrous building blocks, depending on the type of wet-spinning solvent and coagulating medium [15–17]. Depending on the polypeptide chemical treatment and orientation at the molecular scale, resulting fibres can display good wet stability in aqueous environment offering possibilities for the design of textile or nonwoven architectures suited to clinical use. Besides fibre formation, reliable synthetic methods should be applied to the resulting fibrous system so that defined polypeptide-based network architectures can be introduced at the molecular scale, thereby ensuring adjustable mechanical properties and wet-stable morphology [18].

In order to prepare polypeptide fibres with adequate mechanical properties to enable them to retain their structure and function in applicable clinical and biological environments, we investigated the wet-spinnability of gelatin and HFC, and characterised resultant fibre properties in the as-spun and crosslinked states. The effect of the wet-spinning solvent, coagulating medium and polypeptide molecular weight on corresponding fibre morphology was addressed aiming to accomplish homogeneous fibres with controlled morphology. Varied polypeptide-based covalent networks were synthesised in the fibrous state via (i) reaction with activated 1,3-phenylenediacetic acid (Ph) in the presence of carbodiimide (potentially resulting in either intra- or intermolecular crosslinks); (ii) via reaction with activated Ph only; (iii) via carbodiimide-mediated condensation, leading to the formation of intramolecular covalent crosslinks. Reacted fibres were investigated with regard to crosslink density, tensile and swelling properties as well as hydrolytic degradability in order to establish the governing structure–property relationships of the fibrous system.

2. Materials and methods

2.1. Materials

Type A gelatin from porcine skin (175 g Bloom), 2,2,2-trifluoroethanol (TFE), dimethyl sulfoxide (DMSO), acetone, ethanol and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma Aldrich. 1,3-Phenylenediacetic acid (Ph), N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimehylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Alfa Aesar. HFC was kindly provided by Nitta Gelatin India Limited.

2.2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-page)

Gelatin and HFC were dissolved in SDS sample buffer (160 mM Tris–HCl, pH 6.8, 2% SDS, 26% glycerol, 0.1% bromophenol blue) at 1% (w/v) concentration and heated for 2 min at 90 °C. 30 ml of each sample solution were loaded onto 4% stacking gel wells and separated on 15% resolving gels (200 V, 45 min, room temperature). Protein bands were visualised after 60 min staining (0.1 wt.% Comassie Blue, 12.5 vol.% trichloroacetic acid) and 60 min treatment in water. Two gel lanes were employed for each polypeptide and imaged with ChemiDoc™ MP System using ImageLab software version 4.1 (Bio-Rad). Selected bands (band intensity: 3×10^5–13×10^6) were analysed with regard to their molecular weights, so that the number-average molecular weight (M_n) was determined by averaging obtained molecular weight values.

2.3. Viscosity measurements on wet-spinning solutions

Wet-spinning solutions (3–40%, w/v protein) were prepared in either DMSO or TFE via stirring at 37 °C overnight. The dynamic viscosity of resulting solutions was measured using a DV-E Viscometer (Brookfield Viscometers Ltd., Harlow, UK). The solution was filled into a beaker making sure that no air bubbles were present and the solution temperature was constant.

2.4. Formation of wet-spun fibres

Resulting solutions were transferred into a 10 ml syringe having 14.5 mm internal diameter equipped with Ø 0.8 μm needle, which acted as single nozzle spinneret. The collagen solutions were then extruded from the nozzle tip via a syringe pump at a dispensing rate of 12 ml h^{-1} with the nozzle tip submerged in a coagulation bath containing 1 L of either ethanol or acetone at room temperature. Resulting wet-spun fibres were then removed from the coagulating medium and dried separately at room temperature. A nearly quantitative yield (>99 wt.%) of fibre formation was measured with respect to the polypeptide weight used in the wet-spinning solution.

2.5. Scanning electron microscopy (SEM)

Dry samples were fixed on carbon stubs and gold sputtered under argon prior to SEM. The structure and morphology of wet-spun fibres were examined using SEM (JEOL SM-35) at different magnifications. The diameter of each fibre group was quantified from SEM images in six different locations, so that average and standard deviation were used for data representation. The internal pore size in each fibre was quantified in the same manner whereby fifteen measurements were taken per each sample group.

2.6. Crosslinking of wet-spun fibres

Three different crosslinking methods were applied to wet-spun fibres at room temperature. In the first instance, samples were incubated with NHS-activated Ph in the presence of EDC-NHS as follows: 0.074 g of 1,3-phenylenediacetic acid (Ph) was dissolved in 100 ml ethanol with magnetic stirring at 40 °C till a clear solution was obtained. Resulting Ph solution was cooled down to room temperature and 4.5 mmoles of both 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were added. The solution was stirred for 30 min in order to activate Ph carboxylic acid terminations, following which time 0.06 g of fibres was added into the solution for the crosslinking reaction to occur. Alternatively, samples were reacted with NHS-activated Ph following the above procedure with the only difference that an equimolar amount of β-mercaptoethanol (βME) with respect to EDC/NHS was added following Ph activation and prior to the crosslinking reaction, in order to deactivate EDC/NHS. Ultimately, samples were crosslinked via state-of-the art EDC/NHS-mediated condensation reaction (EN), whereby 0.06 g fibre was incubated in 100 ml ethanol solution containing 4.5 mmoles of both EDC/NHS. All reacted samples were collected, rinsed thoroughly with ethanol and air-dried.

2.7. Swelling tests

Swelling tests of the fibres were performed by incubating dry samples in 50 ml PBS for 24 h at 37 °C. PBS-equilibrated samples