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Collagen fibres by thermoplastic and wet spinning

M. Meyer [a,*], H. Baltzer [a], K. Schwikal [b]

[a] Forschungsinstitut fuer Leder und Kunststoffbahnen, Meissner Ring 1-5, D-09599 Freiberg, Germany
[b] Thüringisches Institut für Textil-und Kunststoff-Forschung e.V. Breitscheidstr. 97, D-07407 Rudolstadt, Germany

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ABSTRACT

Collagen threads of high yardage were manufactured by two different techniques being wet spinning of collagen dispersions and melt spinning of thermoplastic collagen. The fibres were characterised according to their structural, textile physical and biochemical properties. The wet spun fibres showed higher physical stability than the thermoplastic spun ones. Both fibre types were cross linked with different agents (formaldehyde, glutaraldehyde, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimid (EDC)) to increase mechanical stability as well as to lower susceptibility against enzymatic attack. Fibres cross linked by 0.1% glutaraldehyde as well as non cross linked fibres showed no cytotoxic effect against mouse fibroblasts.

1. Introduction

Collagen is still one of the most popular biomaterials though synthetic polymers became available for medical purposes in the last decades. Collagen combines very important properties. It is biocompatible, biodegradable, and hemostyptic; its biodegradability is being adjusted by cross linking. In tissue engineering applications the collagen's surface leads to good cell attachment, proliferation and differentiation of cells [1,2]. Therefore, collagen is widely used to manufacture medical devices e.g. membranes and sponges, to coat implants and as a solution for tissue augmentation in plastic surgery and as matrix material for cell culture [2–4].

Collagen is the major component of the extracellular matrix (ECM). Accounting for about 30% of the total body protein in vertebrates it represents the most important part of connective tissue beside water. Collagen comprises a group of different collagen types. Many of them are fibre forming others are building networks. The main fibril forming type in skin, tendon and blood vessels is collagen type I [5].

For most applications collagen is extracted from mammalian tendon or skin (porcine, bovine or equine sources). The usually highly insoluble raw material is treated with acid to obtain acid soluble collagen (ASC) or by pepsin digestion to improve yield (pepsin treated collagen, PSC). The concentration of these highly viscous acidic collagen solutions usually lies between 1% and 3%. In some cases collagen is not extracted from the raw material by dissolution but the raw stock is purified and minced completely several times resulting in collagen dispersions [3,6]. These dispersions show higher solid contents and higher process yields.

Solid collagen devices are usually made from collagen solutions or from purified collagen dispersions by casting and drying at room temperature leading to compact membranes or by lyophilisation leading to porous sponges [7]. These structures are usually undirected. To date it is a challenge to manufacture directed 2D and 3D structures, which could be very interesting for tissue engineering applications as well as for making textile implants.

However, the possibility of making directed structures from collagen solutions and dispersions, respectively is limited. Other than synthetic polymers (polyesters, polyamides, polypropylene, and PTFE) or other natural polymers (silk, cotton) collagen is difficult to be formed into fibres of high yardage, because collagen does not consist of isolable long fibres.

Some trials were performed in the past to manufacture threads from different collagen sources. Cavarallo et al. [8] described a process of precipitation of ASC, extracted from bovine tendon in solutions by polyethylene glycol 8000 followed by washing in a buffering solution and dewatering in isopropanol. The threads were cross linked to achieve suitable stability. In a series of papers Zeugolis et al. [9–12] described the preparation of threads (30 cm) according to a similar technique, and Vasilev et al. [13] showed that spinning of long collagen fibres was possible by extrusion of 1.5%–3% solutions of ASC in precipitation baths consisting of acetone and ethanol. The stability of the spinning process strongly depended on the collagen concentration. In contrast, Chanukov et al. [14] and Bienkiewicz et al. [15] used collagen dispersions prepared by extensive mincing with similar results. However, to date there are no high yardage collagen threads available.

The so far described techniques used aqueous solutions or dispersions of collagen containing dry matter contents of 1% up to 10%.

* Corresponding author.
E-mail address: michael.meyer@filkfreiberg.de (M. Meyer).

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Recently, Meyer et al. [16] published a technique to process partly denatured collagen thermoplastically using a completely dry process. This thermoplastic collagen differs from gelatine by its low solubility in water at >35 °C. However, the thermoplastic collagen is already partly denatured having lost its fibrous structure. This material allows to be processed by the same machines as for processing thermoplastic synthetic polymers using temperature controlled processes.

Our investigation aimed at manufacturing collagen threads with high yardage by two different but simple technologies namely wet spinning of collagen dispersion coupled with precipitation of the material as well as thermoplastic melt spinning. The resulting fibres of several dozen meters of length were characterised regarding their processing properties as well as regarding their structural, textile physical and biochemical properties.

2. Experimental part

2.1. Raw material

Porcine and bovine skins were used as collagen raw stock obtained from a local abattoir. The skins were washed extensively and soaked in a solution of sodium hydroxide at pH 12.5 overnight. The swollen skins were split two times on a conventional splitting machine (Turner 537) used in tanneries. The middle split was soaked in ammonium sulphate solution to adjust pH by 7 and washed again with water.

2.2. Collagen dispersions and wet spinning

The collagen dispersions were prepared from alkaline treated bovine and porcine splits. These splits were treated with 1% H_2O_2 solution, minced by a meat chopper, acidified to pH 4 by hydrochloric acid and further treated in a colloid mill (Cavitron, special design) several times to get homogenous collagen masses. The dry matter content of these masses was 1 to 2%. This dry matter content was too low to obtain stable threads, however. Therefore, the masses were adjusted to pH 5, concentrated by centrifugation on a tube centrifuge (special design) up to a dry matter content of 10% and acidified again by addition of low volumes of 1 M hydrochloric acid. This mass had to be aged for 24 h minimum by storage at 4 °C.

The collagen dispersions were processed to threads by a cylinder spinning system (Biedermann & Wolschendorf OHG; Saalfeld, Germany) with a volume of 6 cm^3 and a maximum force of 10 kN. The spinneret design depended on the experimental conditions. Cylindrical and conical nozzles with diameters between 250 and 500 µm were used. The spinning temperature was fixed in all experiments to 26 °C. The spun primary thread was coagulated in a bath designed as a chute containing ethanol/acetone mixtures at different compositions. The threads were picked up at the end of the chute, air dried by a blow drier and wound up on a bobbin.

2.3. Thermoplastic granules and thermoplastic spinning

According to the principle technology for preparation of thermoplastic collagen the split skins were denatured thermally by soaking for 10 min in boiling water, drained off excess water and loft dried [16]. The pieces were then ground to powder on a centrifugal mill (Görgens Engineering GmbH, Dormagen, Germany). This powder was mixed with 5 to 15% glycerol and 15 to 50% deionised water in a fast mixer resulting in granules to be fed to an extruder.

Melt spinning was performed on a single-screw extruder RCP-0500 (Randcastle Extrusion Systems, New Jersey, US) coupled with a spinning pump, a stretching system consisting of primary rolls, secondary rolls and a heating section and finally take-up rolls. The extrusion temperature was adjusted in the range of 90 to 98 °C depending on the recipe to achieve applicable viscosities. The rotation speed was set between 50 and 120 rpm to get a processing pressure of 50 bar. The spinning pump was adjusted to 10 rpm. The collagen melt was formed to unifilar fibres by extrusion through nozzles with diameters of 0.3 and 0.5 mm.

2.4. Material testing

The fibre parameters such as tensile strength and elongation at break of the fibres were determined with the automatic testing system Fafegraph M (Textechno, Mönchengladbach, Germany) according to DIN EN ISO 5079 with 25 replicates for each sample. The analyses were performed at 20 °C and 60% relative humidity, with a feed of 10 mm/min. The fineness was investigated according to DIN EN ISO 1973.

During SEM-measurements the fibres were fixed on sample holders and were subsequently coated with gold using a magnetron sputter coating device Polaron SC7620 (Quorum Technologies Ltd., Ringmer, UK). The measurements were carried out with a Leica S440i (Leica Microsystems GmbH, Wetzlar, Germany) at 20 kV.

X-ray diffraction was measured on a Bruker D8 Advance diffractometer using CuK_α radiation ($\lambda = 0.154$ nm) in transmission. The generator system operated at 40 kV. The manufactured collagen fibres were prepared for measurement by taking some pieces together in a parallel orientation and fixing them with cellulose nitrate. The aqueous dispersion was brought as film on a glass plate and dried on air.

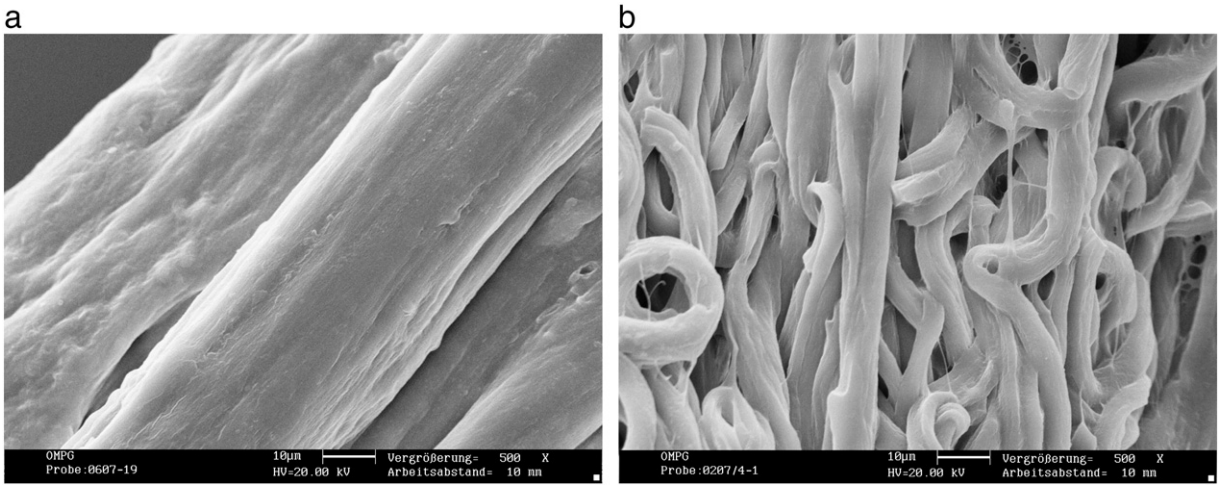

Fig. 1. SEM-measurements of fibres manufactured with a conical spinneret from dispersions of porcine collagen (a) and bovine hide collagen (b) at the same magnification.